

# Supporting Information

## A Fluorescent Label-Free Aptasensor Integrated in a Lab-on-Chip System for the Detection of Ochratoxin A in Beer and Wheat

*Francesca Costantini<sup>‡\*</sup>, Nicola Lovecchio<sup>†</sup>, Albert Ruggi<sup>§</sup>, Cesare Manetti<sup>¥</sup>, Augusto Nascetti<sup>&</sup>  
Massimo Reverberi<sup>‡</sup>, Giampiero de Cesare<sup>†</sup> and Domenico Caputo<sup>†</sup>*

<sup>‡</sup>Department of Chemistry, Sapienza University of Rome, p.le Aldo Moro 5, 00186 Rome, Italy

<sup>†</sup>Department Information Engineering, Electronics and Telecommunications, Sapienza  
University of Rome, via Eudossiana 18, 00184 Rome, Italy

<sup>§</sup>Department of Chemistry, University of Fribourg, 1700 Fribourg, Switzerland

<sup>¥</sup>Department of Environmental Biology, Sapienza University of Rome, p.le Aldo Moro 5, 00186  
Rome, Italy

<sup>&</sup>School of Aerospace Engineering, Sapienza University of Rome, via Salaria 851/881, 00138  
Rome, Italy

\*Corresponding author: email [francesca.costantini@uniroma1.it](mailto:francesca.costantini@uniroma1.it)

## Reagents and Equipment

All reagents were purchased by Aldrich Chemicals. 2-Hydroxyethyl methacrylate (HEMA) was distilled prior to use, whereas the other chemicals were used without further purification. 2-Bromo-2-methyl-propionic acid 3-trichlorosilylanyl-propyl ester (BMPTS) is synthesized following a reported procedure.<sup>1</sup> Acetone, acetonitrile and ethanol 96% (analytical reagent grade) were used without further purification, while toluene was distilled over sodium. Water was purified with a Milli-Q pulse (MILLIPORE, R=18.2 M $\Omega$ ·cm) ultra-pure water system.

Aptamer Amino-1.12.2 (5'-[AmC6T] GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA-3')<sup>2</sup> was purchased by Sigma Aldrich.

Rutenium 1,10-phenatroline dipirido phenazine ([Ru(phen)<sub>2</sub>(dppz)]<sup>2+</sup>) was synthesized according to the reported procedure<sup>3</sup>.

Fluorescence images were taken using a 10X objective (100X total magnification) of the inverted phase contrast fluorescence microscope Leica DMIL, endowed with a 100 W mercury vapor lamp, and a TRITC filter set, and coupled with high resolution and sensitivity CCD photcamera (Jenoptik and Zeiss AxioCam ICc3) driven by Zen 2011 software. Analysis of fluorescence images was performed by Zen 2011 software, all the images were taken using the same exposure time.

## Preparation of the Patterned Glass Slide

The patterned glass slides were obtained with the following procedure. Initially, glass slides were treated with piranha and rinsed with ultrapure water. Subsequently, they were spin-coated with the SU-8 3005 and exposed to UV light using a mask having the pattern of lines (diameter:

100  $\mu\text{m}$ ). The glass slides were soaked for 1 min in a solution of SU-8 developer, rinsed with isopropanol and dried with the stream of nitrogen <sup>4</sup>.

### **Synthesis of the PHEMA Brush Layer and its Functionalization**

The bare glass slides were immersed in a Piranha solution ( $\text{H}_2\text{SO}_4$ :  $\text{H}_2\text{O}_2$  3:1) for 20 min, copiously rinsed with Milli-Q water and then dried with a stream of nitrogen. Subsequently, all the samples were incubated in a solution of 0.01% of 3-trichlorosilylanyl-propyl ester (BMPTS) in dry toluene over the night. The formed self-assembled monolayer was rinsed with dry toluene, acetone and ethanol and finally dried with a stream of nitrogen. After this step, the patterned SU-8 layer was removed immersing the glass slides in a solution of SU-8 developer; afterwards they were rinsed with ethanol and dried with the stream of nitrogen. The PHEMA polymer film was then grown on all the samples using previously reported procedures. Briefly, a solution containing 20 mL of 2-hydroxyethyl methacrylate (HEMA) and 20 mL of water was degassed by bubbling through dry nitrogen ( $\text{N}_2$ ) for 30 min and transferred in a schlenk tube where it was stored under argon. Copper(I) chloride (0.110 g), copper(II) bromide (0.072 g) and 2,2'-dipyridyl (0.488 g) were added. To dissolve the solid, the mixture was stirred for 10 min (while degassing), which yielded a dark brown solution. The solution was then sonicated till complete dissolution of the solid and subsequently transferred using a syringe into a home-made schlenk tube containing all the samples. After polymerization (3 h at room temperature in dark condition), the samples were removed and washed with ethanol and MilliQ water. The PHEMA polymer films were soaked (18 h, at room temperature) in a solution of succinic anhydride (1.5 mg) and triethylamine (1.5 mL) in 27.5 mL of dry tetrahydrofuran (THF), to obtain carboxylic moieties (PHEMA-SA).

## **Fabrication of the Photosensor Array**

The bottom electrode is a 180 nm-thick Indium Tin Oxide (ITO) layer grown in a magnetron sputtering system purchased from Materials Research Corporation (Orangeburg, NY, USA), while the top metal electrode is a three-metal layer stack (30 nm-thick Cr/150 nm-thick Al/30 nm-thick Cr) deposited in the same sputtering system. The a-Si:H layers have been deposited by Plasma Enhanced Chemical Vapor Deposition (PECVD) in a three-chamber high vacuum system (Glasstech Solar Incorporation, Denver, Colorado). The optoelectronic properties of the photosensors have been inferred through their current voltage curves (IV) in dark conditions and their quantum efficiency (QY) response, before and after the filter deposition. The IV characteristics were measured by using a Keithley 236 Source Measure Unit (SMU) in mode voltage-source/current-measure. The QY measurements have been performed by using a set-up which includes a tungsten light source, a monochromator (model Spex 340E from Jobin- Yvon), an UV-enhanced crystalline silicon diode (model DR 2550-2BNC from Hamamatsu) used as reference, a beam- splitter and focusing optics (from Melles-Griot).

The fabrication steps of the whole a-Si:H photosensor array are:

- cleaning of the glass substrate;
- deposition of the ITO layer by Reactive Ion Etching (RIE) in an IONVAC system (Pomezia, Rome, Italy) and its patterning through photolithographic process;  
deposition of the a-Si:H layers p-type/intrinsic/n-type with thickness equal to 10, 400 and 50 nm, respectively;
- deposition of the three-metal Cr/Al/Cr stack;
- wet etching of the Cr/Al/Cr stack and RIE etching of the a-Si:H layers for the photolithographic patterning of the diodes;

- deposition by spin coating of a 5  $\mu\text{m}$ -thick SU8-3005 (from Micro-Chem, MA, U.S.A.) and its patterning for opening via holes over the diodes;
- deposition by sputtering of a 150 nm-thick titanium/tungsten alloy layer and its patterning for the connection of the top electrodes to the pads;

Further details on the deposition parameters and photolithographic recipes utilized in the photosensor array can be found in a previously reported publication <sup>4</sup>. It is important noticing that top electrode is shared through all the diodes, while the bottom electrode is independent for each diode. The area of each diode is  $2 \times 2 \text{ mm}^2$ , while the pitch of the array is 4 mm.

### **Filter fabrication and characterization**

The long-pass interferential filter has been designed with a free-ware software, XOP, using the IMD extension [<http://ftp.esrf.fr/pub/scisoft/xop2.3/>]. The interferential filter is a stacked structure of alternating layers of zinc sulfide (ZnS) and magnesium fluoride ( $\text{MgF}_2$ ) uniformly deposited over the borofloat glass substrate already hosting the photosensor array. In order to perform the optical characterization, the filter is deposited also on plain borofloat substrates. The raw grains of ZnS and  $\text{MgF}_2$  for the filter deposition is purchased from Umicore Thin Film Products AG (Balzers, Liechtenstein). A cross section of the filter structure is reported in Figure 1b. The dielectric layers of ZnS and  $\text{MgF}_2$  are fabricated by Electron Beam Physical Vapor Deposition (EB-PVD) in a BAK-6040 Balzers system. The deposition occurs at room temperature; the initial vacuum pressure is set at  $10^{-8}$  Torr, while the working pressure at  $10^{-5}$  Torr. The distance between the sample and the crucible is around 50 cm. The thickness uniformity is enhanced by the sample rotation through a cage inside the evaporation system. A preliminary calibration allowed to determine the deposition rate of the two materials. Filter

reflectance and transmittance spectra are measured by a Lambda 950 spectrophotometer from Perkin Elmer (Waltham, Massachusetts, USA) on the plain borofloat glass substrates.

## References

(1) Costantini, F.; Bula, W. P.; Salvio, R.; Huskens, J.; Gardeniers, H.; Reinhoudt, D. N.; Verboom, W. Nanostructure Based on Polymer Brushes for Efficient Heterogeneous Catalysis in Microreactors. *J. Am. Chem. Soc.* **2009**, *131* (5), 1650-1651.

(2) McKeague, M.; Velu, R.; De Girolamo, A.; Valenzano, S.; Pascale, M.; Smith, M.; DeRosa, M. C. Comparison of In-Solution Biorecognition Properties of Aptamers against Ochratoxin A. *Toxins* **2016**, *8* (11), 1-13.

(3) Jiang, Y.; Fang, X.; Bai, C. Signaling Aptamer/Protein Binding by a Molecular Light Switch Complex. *Anal. Chem.* **2004**, *76*, 5230-5235.

(4) Costantini, F.; Sberna, C.; Petrucci, G.; Reverberi, M.; Domenici, F.; Fanelli, C.; Manetti, C.; de Cesare, G.; DeRosa, M.; Nascetti, A.; Caputo, D. Aptamer-based sandwich assay for on chip detection of Ochratoxin A by an array of amorphous silicon photosensors. *Sens. Actuat. B-Chem.* **2016**, *230*, 31-39.