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5 **Title:** An amorphous silicon photodiode microfluidic chip to detect nanomolar quantities of  
6 HIV-1 virion infectivity factor  
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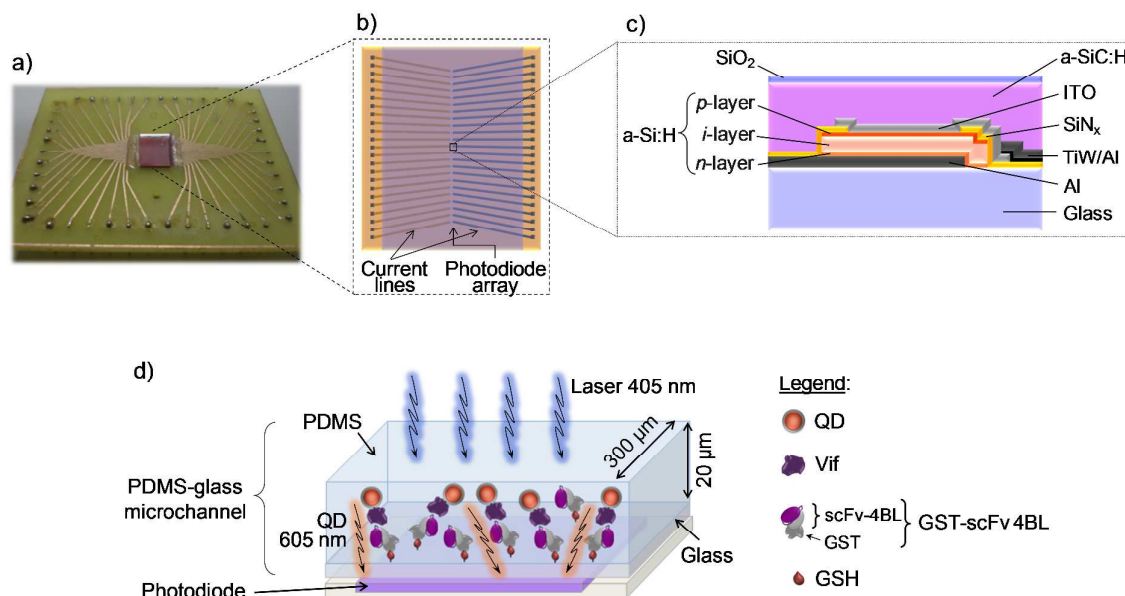
**Abstract**

Hydrogenated amorphous silicon (a-Si:H) photosensor was explored for the quantitative detection of HIV-1 virion infectivity factor (Vif) to a detection limit in the single nanomolar. The a-Si:H photosensor was coupled with a microfluidic channel that was functionalized with a recombinant single chain variable fragment antibody. The biosensor selectively recognized HIV-1 Vif from human cell extracts.

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Semiconductor technology has been used to develop integrated photosensors containing the critical components for optical detection integrated on a single chip. Such integrated chips have been shown suitable for the sensitive, rapid and real-time measurement of optical signals,<sup>1,2</sup> also leading to portability and reduced costs, which are the major factors in boosting the miniaturized biosensors uptake in the healthcare sector particularly for on-site medical diagnostics uses.<sup>3</sup> Hydrogenated amorphous silicon (a-Si:H) photosensor have been described as a portable and sensitive detection platform, offering a promising alternative to conventional optical analytical methods.<sup>4,5</sup> In particular, a-Si:H photodiodes fit perfectly for the use as integrated photosensors because of their high quantum efficiency at visible wavelengths, low dark current, suitability for low-temperature (below 250 °C) processing technology which allow the use of a wide variety of inexpensive substrates for chip manufacturing such as glass, plastic and polymers, and easy integration into chip.<sup>4,5</sup>

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In this work, HIV-1 proteins in solution are detected by an a-Si:H photosensor, which contains an integrated a-Si:H PIN photodiode element to convert incident light into a measurable electrical current, and an optical filtering layer to discard the effect of undesired wavelengths from the excitation light. The target protein is the HIV-1 virion infectivity factor (Vif), a 23 kDa protein mainly found in the cytoplasm of HIV-1 infected cells,<sup>6</sup> which was identified as a potential target in antiviral therapy.<sup>7</sup>

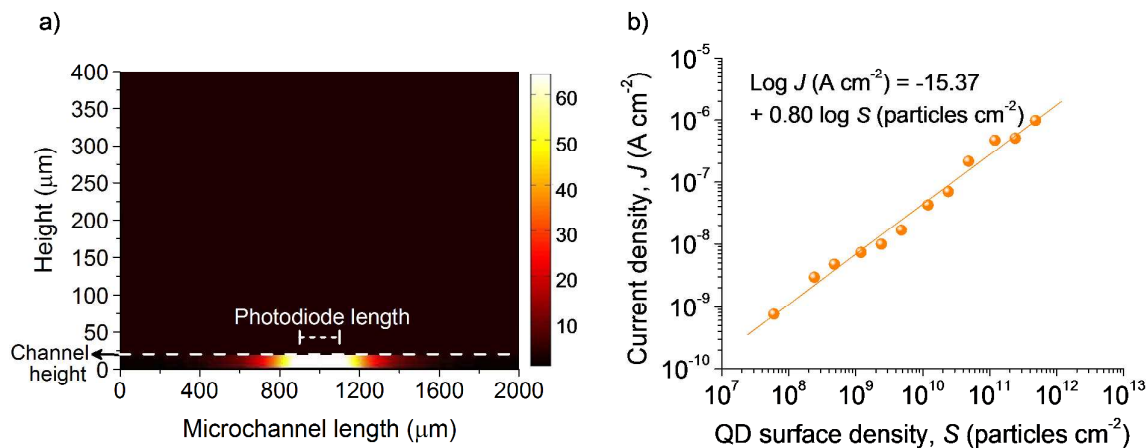
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As schematically represented in Fig. 1a,b,c, the chip consists of an array of twenty three 200  $\mu\text{m} \times 200 \mu\text{m}$  a-Si:H PIN photodiode sensors integrating an amorphous silicon carbon (a-SiC:H) filter.<sup>5</sup> The chip was microfabricated as described elsewhere<sup>2,5</sup> using radio frequency plasma enhanced chemical vapor deposition and reactive ion etching to pattern the device a-Si:H PIN photodiode islands. The photodiode was deposited above the bottom contact aluminum (150 nm) and is formed by 20 nm *n*-type a-Si:H, 500 nm intrinsic a-Si:H, and 20 nm *p*-type a-Si:H. An 100-nm insulating layer of silicon nitride ( $\text{SiN}_x$ ) is used as a sidewall passivation layer and allows electrical contact between the transparent 100-nm indium tin oxide (ITO) top electrode with *p*-type a-Si:H layer. Titanium tungsten (15 nm)/aluminum (150 nm) (TiW/Al) are defined as top contact lines. A 1.96  $\mu\text{m}$  thick a-SiC:H filter film with a 2.25 eV band-gap is used to block the excitation light and to transmit the fluorescent emission light to the a-Si:H. Silicon dioxide (75 nm) was used as a passivation layer to protect the chip.



**Fig. 1** a-b) The 23-photodiode sensor chip mounted on a printed circuit board. c) Cross sectional schematic view of the a-Si:H PIN photodetector. d) Schematic of the biorecognition reactions of the probe (GST-scFv 4BL) with the target protein (Vif) labeled with quantum dots (QD) within the microchannel. a-SiC:H, amorphous silicon-carbon filter; ITO, indium tin oxide top contact, Al, aluminum bottom contact; SiN<sub>x</sub>, silicon nitride insulator film; SiO<sub>2</sub>, silicon dioxide insulator film.

The photodiodes were mounted underneath a microfluidic channel to generate the biosensor device. The microfluidic chip consists of a PDMS channel with 300  $\mu\text{m}$  in width, 20  $\mu\text{m}$  in height and 2 mm in length with one inlet and one outlet port. The channel was constructed using standard SU-8 molding and mounted on a glass slide by UV-ozone treatment sealing. The microchannel chip was functionalized with a recombinant single chain variable fragment (scFv) antibody fused to glutathione S-transferase (GST) – GST-scFv 4BL – and used to detect the target protein previously labeled with carboxyl quantum dots (QDs) 605 ITK (Invitrogen). A scheme of the use of the biosensor chip for the detection of the target proteins is shown in Fig. 1d.

The optoelectronic characteristics of the a-Si:H PIN photosensor chip<sup>8</sup> for the wavelengths of interest (405 and 605 nm) are shown in Supplementary Information. These wavelengths were chosen because they correspond respectively to the excitation and emission wavelengths of the selected QDs to fluorescently label the target HIV-1 Vif protein.



**Fig. 2** a) Scheme derived from the optical model demonstrating the zones of photons emitted by the QDs, within the microchannel, that diffuse to the detector. b) Photoresponse of the a-Si:H photosensor plotted as a function of the surface density of QDs.

As the objective of the biosensor chips is the detection of biomolecules labeled with QDs, an estimation of the amount of fluorescent light and the calibration of the photodiode response to increasing concentrations of QDs was needed. As such, the fraction of photons ( $\phi$ ) emitted by the QDs within the microfluidic channel that reaches the photodiode sensor, and thus generates a signal, was estimated from a model based on the isotropy of the emitted light.<sup>9</sup> The optical model described in Pimentel *et al.*<sup>9</sup> relates the ratio of fluorescence collected by the photodiode with the geometry of the photodiode and the relative position between the emitting species (QDs) and the photodiode. The fluorescence signal detected by a photodiode with side length  $l = 200 \mu\text{m}$ , located at a distance  $L$  from a layer of fluorescent species with a determined dimension is directly proportional to the fraction of photons reaching the device,  $\phi$ . Assuming that the photons are emitted from a distance  $L = 175 \mu\text{m}$  of the photodiode sensor, which is the average thickness of the glass used for the microfluidic chips, the fraction of photons emitted isotropically by the QDs inside the microchannel that reach the photodiode chip is  $\phi = 1.4 \times 10^{-2}$ .

In Fig. 2a is represented the scheme derived from the model that demonstrates the zones of emitted photons that reach the detector from QDs within the microchannel, as a function of the position of the photodiode (indicated as photodiode length on the scheme), and therefore contribute to the sensor signal. From all QDs within the microchannel, the main contribution to the photodiode response is given by the QDs that are over the sensor chip, as represented by the white zone in the microchannel scheme in Fig. 2a.

The photodiode signal was calibrated using solutions of 0.1 to 800 nM of QDs in Milli-Q water. The microfluidic channels were filled with the QD solutions and the sensor

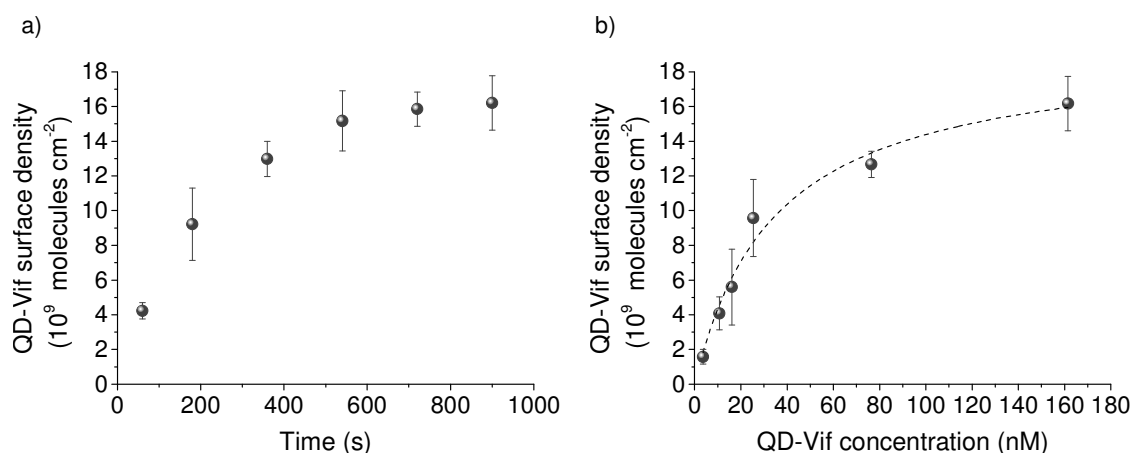
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3 photocurrent was measured after excitation with 405 nm light. The measured photocurrent  
4 was considered the fluorescence signal after removing the background signal obtained when  
5 only Milli-Q water was used. As represented in Fig. 2b, the photodiode mounted underneath  
6 the microfluidic channel chip responds linearly to the increasing concentration of QDs. The  
7 limit of detection (LOD), which establishes the lowest measured signal that can be considered  
8 significant, was set as 3-fold the standard deviation of the noise signal. For the biosensor  
9 chips herein used,  $LOD = 5.0 \times 10^7$  QDs  $cm^{-2}$  which corresponds to 80 pM was determined –  
10 the surface density of QDs was estimated by dividing the total number of QD particles in  
11 solution by the area of the microchannel top and bottom surfaces.

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18 The functionalization of the biosensor chip, thus the immobilization of the bioreceptor  
19 molecules on the sensor surface, is a key step for the development of any biosensor.<sup>10</sup> The  
20 optimal functionalization approach must ensure high sensitivity and selectivity, which are  
21 accomplished by maximizing the density, activity and stability of the bonded bioreceptor, and  
22 by minimizing non-specific adsorption. The surface chemistry used to modify the sensor  
23 surface and the selected ligands have a critical role.<sup>10</sup> We have recently optimized a procedure  
24 to modify glass chips with silanes in which an optimal mixture of 2.5% of  
25 3-mercaptopropyltrimethoxysilane (MPTS) and a post-silanization curing process are used to  
26 maximize the surface density of chemically active thiol functions and to minimize  
27 non-specific adsorption of proteins.<sup>11</sup> After modifying the surface of the microfluidic channels  
28 with MPTS, glutathione (GSH) was covalently immobilized by incubating during 30 min a  
29 solution of GSH 33 mM in PBS pH 7.0 to generate the GSH-functionalized chips. GSH is  
30 known to enable the immobilization of biomolecules containing a GST domain via specific  
31 GST/GSH interactions.<sup>12-14</sup> On the other hand, GST is a very common domain used to label  
32 proteins by fusion techniques in molecular biology applications. We explore this GST/GSH  
33 interaction to generate a biosensor surface with highly ordered orientation of the immobilized  
34 bioreceptors. Recombinant scFv antibody – scFv 4BL – previously generated against HIV-1  
35 Vif protein<sup>15</sup> was fused to the affinity tag GST, expressed in *Escherichia coli* and purified as  
36 described in Supplementary Information. The GST-scFv 4BL fusion was further immobilized  
37 on the GSH-functionalized microchannels.

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The performance of the antibody-functionalized microchannels to recognize Vif was evaluated with the a-Si:H photosensor. Purified Vif protein<sup>16,17</sup> was labeled with QDs by conjugation using standard EDC/NHS coupling chemistry. A phosphate buffer solution of Vif-QD conjugates was flowed into the microchannel at a flow rate of 0.5  $\mu$ L  $min^{-1}$  for 15 min. A diode laser at 405 nm was used as excitation light source and the QDs fluorescence

emission at 605 nm was measured by the a-Si:H photosensor. The current density calibration of the sensor was further used to estimate the density of emitting QDs at the sensor surface. As shown in Fig. 3a, the sensor signal intensity increases as the target HIV-1 Vif protein (conjugated with the QDs) diffuses and binds to the scFv 4BL immobilized at the sensor surface, saturating after 600 s with a measured surface density of  $1.6 \times 10^{10}$  QD-Vif molecules  $\text{cm}^{-2}$ . This surface density is statistically significant different from the QD-Vif surface density obtained for the control experiment of the non-specific interaction of Vif-QD with immobilized BSA on the microchannel –  $1.1 \pm 0.2 \times 10^9$  molecules  $\text{cm}^{-2}$  (*t*-test, *P* < 0.001). The effect of the initial concentration of HIV-1 Vif-QD conjugate in the flowing solution was also investigated. A saturation curve was measured (Fig. 3b) which was fitted to a Langmuir isotherm model to obtain the equilibrium dissociation constant  $K_D = 34 \pm 8$  nM and a maximum surface density of bonded QD-Vif  $S_{max} = 1.9 \pm 0.2 \times 10^{10}$  molecules  $\text{cm}^{-2}$  ( $R^2$  of 0.97).



**Fig. 3** a) Surface density of captured QD-Vif by the immobilized GST-scFv 4BL as a function of flow time of QD-Vif solution. b) Surface density of captured QD-Vif by the immobilized GST-scFv 4BL as a function of the initial concentration of QD-Vif solution flowed into the channel. The dashed line is a fit to the experimental results using the Langmuir equation ( $S_{max} = 1.9 \pm 0.2 \times 10^{10}$  molecules/ $\text{cm}^2$ ,  $K_D = 3.4 \pm 0.8 \times 10^{-8}$  M,  $R^2 = 0.97$ ). The error bars are calculated from the measurements on three different samples.

The value obtained for the dissociation equilibrium constant in the nanomolar range is comparable with other affinity pairs using recombinant antibodies described in literature.<sup>18-20</sup> Moreover, when compared with previously published data obtained for the same affinity pair (scFv 4BL-Vif) on a quartz crystal microbalance immunosensor<sup>17</sup>, the  $K_D$  obtained in this work is two orders of magnitude lower, which is the result of both the higher sensitivity of the a-Si:H photosensor and of the oriented immobilization of the scFV 4BL obtained exploring the GST/GSH affinity for the immobilization, leading to higher availability of recognition



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3 sites. The lower surface density of QD-Vif captured by the immobilized GST-scFv 4BL  
4 detected with the a-Si:H photosensor was  $1.6 \times 10^9$  molecules  $\text{cm}^{-2}$  corresponding to a  
5 QD-Vif concentration in solution of 3.6 nM, also two orders of magnitude lower than the 0.3  
6  $\mu\text{M}$  detected with the previously published QCM immunosensor.<sup>16</sup>  
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10 The transport of target molecules to the surface of the activated sensor chip is a very  
11 important parameter in microfluidic chips.<sup>21,22</sup> The designed microfluidic channels must  
12 indeed ensure that enough analyte molecules are transported to the surface of the sensor  
13 where they can bind to the immobilized receptors and generate the detected signal.<sup>21,22</sup>  
14 Convection and diffusion are the two mass transport mechanisms involved in the transport of  
15 analyte molecules to the sensor surface.  
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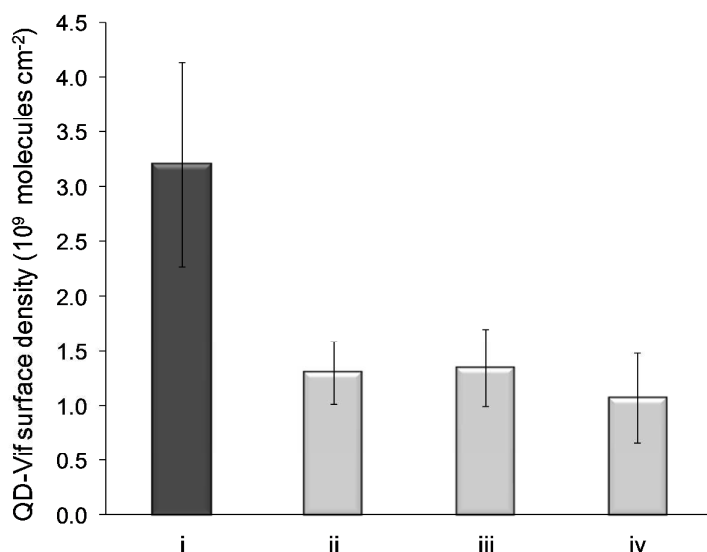
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19 The relative importance of mass transport by diffusion and by convection is  
20 characterized by the Péclet number,  $Pe = Q/DW$ , where  $Q$  is the flow rate,  $D$  is the diffusion  
21 coefficient of the analyte and  $W$  is the channel width. For  $Pe \ll 1$ , diffusion is faster than  
22 convection which in practical terms means that the amount of analyte reaching the sensor  
23 area, thus reacting with the immobilized biomolecules, depends on the renewal rate of the  
24 feed solution stream. On the other hand, for  $Pe \gg 1$ , convection is faster than diffusion, and  
25 analyte molecules are swept away downstream of the sensor surface before they diffuse across  
26 the channel. The diffusion coefficient for the 20-nm QD conjugate spherical particles is  
27  $2.0 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$  as estimated from the Stokes-Einstein equation. It is thus likely that the  
28 target Vif molecules labeled with the QDs will diffuse very slowly to the sensor. In fact, the  
29 Péclet number for our system at a flow rate of  $0.5 \mu\text{L min}^{-1}$  and a channel width of  $300 \mu\text{m}$ , is  
30  $Pe = 1.3 \times 10^3$ . Convective transport thus dominates over diffusive transport which means that  
31 most of the analyte molecules (bonded to the QDs) are transported away by the flow before  
32 they can diffuse to the sensor surface. Nevertheless, the few analyte molecules reaching the  
33 sensor area within the microfluidic channel will then diffuse to the sensor surface where they  
34 can react with the immobilized antibodies. In this regard, it is important to engineer the  
35 microfluidic system so that the assay is reaction limited, to guarantee that the detection is  
36 governed by the binding kinetics and that the surface concentration of analyte can be  
37 approximated to the concentration in solution. It is thus critical to compare the diffusion time  
38 versus the reaction time in order to understand if the overall binding rate is limited by the  
39 transport of analyte or by the kinetics of the binding reaction. This comparison is given by the  
40 Damköhler number,  $Da = (k_{on} S_{max} H) / D$ , where  $k_{on}$  is the association rate constant and  $H$  is the  
41 channel height.<sup>21</sup> For  $Da \gg 1$ , the system is transport limited meaning that convection and  
42 diffusion deliver analyte molecules to the sensor surface area so slowly that the binding  
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3 reaction can be considered instantaneous. For  $Da \ll 1$ , molecules are transported to the sensor  
4 surface faster than they can bind and the process is reaction limited. Using a dissociation rate  
5 constant ( $k_{off}$ ) previously determined using piezoelectric biosensors for the same  
6 scFv 4BL-Vif affinity pair<sup>17</sup> –  $k_{off} = 1.1 \times 10^{-3} \text{ s}^{-1}$  – the association rate constant  
7  $k_{on} = 3.2 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$  was calculated from the equilibrium constant ( $K_D = k_{off}/k_{on}$ ) obtained in  
8 this work (see above). The Damköhler number is  $Da = 9.7 \times 10^{-3}$ , which shows that this  
9 system operates in the reaction-limited regime and so the transport of analyte molecules to the  
10 sensor surface is fast enough to deliver sufficient target molecules over the surface. No  
11 analyte depletion occurs and the sensor can be used to detect and quantitatively measure the  
12 presence of HIV-1 Vif.  
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20 To explore the feasibility of monitoring HIV-1 Vif using the integrated photodetector  
21 in real-life situations, we evaluated the detection of target analyte in human cell extracts. For  
22 this assay, the HIV-1 Vif produced by human embryonic kidney cell (HEK 293T) cultures  
23 was expressed as a fusion protein with the cyan fluorescent protein (CFP) to enable the  
24 monitorization of the Vif expression during cell culture by the fluorescence of the fused CFP.  
25 Not transfected cells and cells expressing only the CFP protein were used as controls.  
26 Expressed proteins were extracted from the cells after 48 h of culture and conjugated to QDs  
27 as described in Supplementary Information.  
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33 The amount of HIV-1 Vif present in the cell extracts was  $0.15 \mu\text{M}$ , which corresponds to  
34  $9.0 \times 10^{13} \text{ molecules cm}^{-3}$ , as quantified from the CFP fluorescence measured on a Tecan  
35 Infinite M200 microplate fluorescence reader. The quantification of the total protein present  
36 in the cell extracts revealed that Vif only accounts for 0.4% of the total protein, which  
37 demonstrates the complexity of the sample. After conjugation with QDs (in which cell  
38 extracts were diluted at the concentration required for stoichiometric 1:1 Vif:QDs  
39 equivalence), the GST-scFv 4BL-functionalized microchannels were challenged with  
40 QD-labeled cell extracts. The concentration of QD-labeled cell extracts that initially flows  
41 into the microchannel, as also measured by the photosensor, was  $\sim 85 \text{ nM}$ , roughly  
42 corresponding to  $0.34 \text{ nM}$  of Vif. Microchannel chips functionalized only with GSH,  
43 therefore with no GST-scFv 4BL, were also prepared and used as control for the non-specific  
44 binding of the QD-labeled cell extracts from cells expressing Vif. As shown in Fig. 4, the  
45 detected surface density of QD-Vif from cell extracts expressing Vif was  
46  $3.2 \times 10^9 \text{ molecules cm}^{-2}$ , almost three times higher than the density obtained for the  
47 non-specific reactions with the control cell extracts –  $\sim 1.3 \times 10^9 \text{ molecules cm}^{-2}$  ( $t$ -test,  
48  $P < 0.05$ ). These results thus show the specificity of the detection and also the capability of  
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3 this microfluidic photodiode biosensor to selectively recognize HIV-1 Vif from complex  
4 cellular mixtures.  
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**Fig. 4** Surface density of QDs obtained for the reaction of immobilized GSH/GST-scFv 4BL in the microchannel with (i) Vif-QD conjugate from cell extracts of HEK 293T expressing Vif, (ii) QDs conjugated with cell extracts obtained from cells only expressing CFP, (iii) QDs conjugated with cell extracts obtained from non-transfected cells. (iv) The non-specific reaction of immobilized GSH with Vif-QD from cell extracts expressing Vif is also shown. The error bars are calculated from the measurements on three different samples.

In summary, a microsensors, which comprises an a-Si:H PIN photodiode with an integrated a-SiC:H filter and an detachable microchannel where the biomolecules are immobilized, was used to optically detect and quantify HIV-1 Vif labeled with QDs. The influence of the initial concentration of QD-Vif in solution on the final captured analyte by the GST-scFv 4BL-functionalized surface was also investigated with the a-Si:H photosensor. The minimum surface density of QD-Vif captured by GST-scFv 4BL was  $1.6 \times 10^9$  molecules  $\text{cm}^{-2}$  which corresponds to a solution concentration in the single nanomolar range. The binding of QD-Vif with the immobilized GST-scFv 4BL followed the Langmuir model with  $K_D = 3.4 \times 10^{-8}$  M. The evaluation of detection of target analyte in complex mixtures was also performed; the methodology imposed was effective in the specific detection of HIV-1 Vif in human cell extracts. The results demonstrated the potential use of the a-Si:H photosensor as an innovative miniaturized biosensor for the diagnostics/monitoring of HIV.

## Acknowledgements

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## Table of Contents

Hydrogenated amorphous silicon (a-Si:H) photosensor is explored for the quantitative detection of HIV-1 virion infectivity factor (Vif) to a detection limit in the single nanomolar. The a-Si:H photosensor is coupled with a microfluidic channel that is functionalized with a recombinant single chain variable fragment antibody. The microfluidic biosensor selectively recognizes HIV-1 Vif from human cell extracts.

