Silicon thin film transistor-based aptamer sensor for COVID-19 detection

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Abstract

Since the beginning of the coronavirus disease 2019 (COVID-19) in December 2019 and the current lack to date of specific drugs or vaccinations to cope with the disease, it has become apparent that the surest way of dealing with this is through early diagnosis and management. Current testing has shown to be unable to rapidly and accurately provide the results required to restrict the spread. Here we report the use of an intrinsic silicon thin film transistor functionalised with aptamers designed to attach to the spike protein of COVID-19. It is shown that a linear response can be obtained in a concentration of 10 fM and 10 pM, and that the relative response is independent of the applied potential allowing a sensory system to fine tune the applied potential to facilitate the interpretation of the results.

Introduction

In December 2019, a number of unusual cases of pneumonia of unknown origin were reported in Wuhan in the Hubei province of China [1]. This was subsequently identified as the novel coronavirus (2019-nCov) and renamed severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) by the International Committee on Taxonomy of Viruses [2]. From early 2020 human-to-human transmission has accelerated the spread of the virus across the world, resulting in the World Health Organisation (WHO) declaring the COVID-19 outbreak as a pandemic in March 2020. By early August 2020 there were over 20 million confirmed cases and over 730,000 deaths related from COVID-19 world-wide [3]. During the first six months of 2020 months, nearly all countries have imposed lockdowns and restrictions upon their citizens to slow the spread of COVID-19 to manageable levels and prevent health services from being overwhelmed. While the severity of these lockdowns and restrictions vary between countries, governments which have deployed mass rapid testing of citizens have generally required less severe restrictions and have appeared to be able to manage outbreaks with fewer cases and fatalities.

Traditionally the presence of *viruses* has been determined by culture followed by observation. Although the advantages of this technique are that it is cheap and has high specificity, problems arise in that the samples need to be taken from the infected area. In order to address these issues, two different types of detection methods have been developed. The first are the so-called antigen tests. The second method is based on molecular based detection [4]. One such technique is polymerase chain reaction (PCR) which is used to amplify the quantity of species-specific genes unique to a disease. While PCR has been used as the gold standard testing method for COVID-19 monitoring, it is time consuming requiring dedicated laboratories, bespoke equipment and trained personal, making it non- ideal for the level of mass testing that is required for COVID-19. As such there is a need to develop faster and simpler testing, whilst maintaining the selectivity and sensitivity offered by PCR.

SARS-CoV2 is highly similar to the SARS virus attributed to the outbreak in 2003 [5]. In general a mature COVID-19 virus has polyprotein, four structural and five accessary proteins [6]. The polyprotein is the open reading frame 1a and 1b, Orf1ab, while the structural proteins are the envelope protein, E, membrane protein, M, nucleocapsid protein, N, and the spike protein, S. The accessary proteins are Orf3a, Orf6, Orf7a,

Orf8 and Orf10. The Orf1ab protein causes the viral RNA replication and transcription [6]. Proteins E and M are responsible for the viral assembly of the coronavirus, while protein N is required for the viral RNA synthesis [7]. Protein S sits on the surface of the viral particle and facilitates the infection of host cells by binding to its receptor, ACE2, via the proteins receptor binding domain. This protein is also the only one that is significantly different to those found in the original SARS-CoV-1 [8].

Over recent years, so-called the immuno-FET, has been demonstrated as a potential low cost bio sensor platform. An immuno-FET is a standard thin film transistor in which the gate is replaced by a layer of antibodies specific for the target protein [9, 10]. Once the target is attached to the antibody, the charged areas of the protein will cause an electrostatic change in the conduction channel of the FET and hence modulate the source-drain current. Such a device and concept has already been demonstrated for potential COVID-19 detection [11].

Currently a major limitation in the performance of immuno-FETs is the screening effect when employed in physiological fluids [12]. The ions present in the solution cause the formation of a double layer with a thickness equal to the Debye length. Any change in the charge distribution outside this layer will not affect the conduction channel of the FET. For a standard salt concentration of 0.1 M, the Debye length is approximately 1 nm. As the antibodies have a size of roughly 10 nm, therefore the target protein cannot be detected by the immuno-FET.

In recent years, a new group of molecules has been developed. These so-called aptamers consist of short DNA, RNA or peptide strands [13, 14]. Utilising their conformation and charge distribution, the aptamers are capable of very specific binding to individual proteins. As they are significantly smaller than the antibodies, the result is that a protein bound via an aptamer to the conduction channel will create the required change in the electrical properties at higher salt concentration. This in turn means that it becomes possible to use this system with physiologically relevant liquids. In addition, aptamers are significantly cheaper and more stable than antibodies.

Within this paper we will look at the response of a basic intrinsic silicon TFT functionalised with an aptamer sequence which binds to the spike protein of COVID-19. Traditionally aptamer functionalised transistors rely on measuring a single source drain potential to determine the target protein concentration dependency. Our focus is to use a wide range of potentials to obtain a broader concentration dependency not only to optimise the response of the sensor, but also to learn more about the processes that are occurring in the binding of the protein by the aptamer.

Results And Discussion

Apart from the required selectivity and sensitivity, a sensory system to be used by a non-trained person must produce a response clear enough to allow an outcome which is unambiguous and easy to interpret. Most systems reported in the literature [11, 15], use a small source drain potential to determine the

response of the system. These small potentials result in relatively small shifts in the source drain current, which is used to detect the presence of the target. This is not an issue when using scientific equipment, but when used without, for example in a point of care application, the variation needs to be improved.

As such it is necessary to look at the IV-curve over a wide range of potentials to determine the most suited within a device. This is shown in Figure 2. It can be seen that there is significant variation in the current levels dependent on the concentration of the spike protein.

A number of distinct features can be observed in Figure 1. There is an initial rapid increase in current, which levels off at about ±1 V, which can be associated with the time dependence of the sweep. After this increase there is a constant increase in current. On the return of the potential towards zero, the current stays constant or even shows a small increase before returning to the same current value at zero. This hysteresis can be explained by the fact that when the potential is increased more trapped states become accessible and fill with electrons.

We make the following correction for experimental variation between measurement runs and samples. The relative change of the current is used to create a device-independent parameter, *R*, defined as

$$R = \frac{I - I_0}{I_0}$$

where *I* is the measured current and I_0 is that measured at the same voltage in the IV characteristic, determined using pure PBS without the addition of the protein. In Figure 2a the ratio R is shown over the whole voltage range for the various concentrations. It is worth noting that R is constant for the returning sweep, and independent of the applied source drain potential.

The constant R, is an important observation as unlike other aptamer or antibody-based transistor sensors where the source drain potential is kept at a maximum of 100 mV [11, 15, 16], here a range of voltages was investigated. At low potentials, a wide variation of *R* can be seen as effectively, the numbers are small. At higher potentials, the ratio remains fairly constant, arising from large differences in absolute numbers, which is easier to differentiate.

Figure 2b shows the source drain current as a function of concentration. The main observation from this figure is that there is a significant variation, up to orders of magnitude, in the current levels as function of the concentration with an almost linear response between the 10 fM and 100 pM. Perhaps surprisingly, an increase in concentration of the spike protein causes the current to reduce compared to the zero concentration values. This effect has been observed before for the case of molecular detection using an indium oxide thin film transistor functionalised with aptamers [15] and was explained by the changes in conformation of the molecules within the aptamer strand. These conformational changes result in a modification of the local electric field and the effect can create either an increase or decrease of the film

resistance. While the situation here is somewhat different in that a protein is detected rather than a molecule, it is not unreasonable to expect that there are certain similarities.

The starting position is an aptamer, which has an inherent charge distribution which is a key parameter for ensuring selectivity in the binding between aptamer and protein. This charge distribution will create a specific resistivity of the intrinsic silicon channel. While it has been reported [17] and proven by the response of the TFT sensor that the aptamer binds to the spike protein, what is unknown is the exact binding location of the protein and how the local charge distribution of the protein is arranged. The observed effect could be explained if the presence of the protein were to limit or shield the effect of the charges on the aptamer, while not adding any additional charge contribution of its own.

Another observation that can be made is that the current variation as a function of concentration spans the whole range of currents. The maximum current is observed when the samples are immersed in a 0 M spike protein solution, or pure PBS. At the maximum detectable concentration, the current decreases to the same level of that of a bare silicon TFT indicating that at that stage, there is no net charge in close proximity to the conduction channel.

A direct comparison with other proteins is not possible as the exact charge distribution near to the silicon channel is unknown and highly likely to be completely different. However it is interesting to compare our results with reported work on a graphene TFT binding to the same spike protein [11]. There are two major differences in the experimental technique compared to the results presented here. The first is that the experiments were performed in liquid, the second is that an antibody was used for the attachment of the spike protein. When comparing the like for like results, the response of the aptamer-based system reported here, was significantly larger than that of the anti-body based system. This difference is related to the afore-mentioned Debye length, which governs the screening distance for the charges of the protein which in turn, modules the conduction channel. The Debye length of both experiments is approximately 1 nm, hence a protein that is further away from the channel due to being bound by an antibody will create less of a response.

Conclusions

In summary, using a known aptamer sequence that binds to the spike protein, we have shown that it can successfully detect its presence using a thin film intrinsic silicon transistor with a detection range between 10 fM and 10 pM or 7.5 pg/ml to 7.5 ng/ml. It has also been shown that the relative change in detection current is fairly constant over the potential range studied here. The study demonstrates that it is possible to obtain a relatively large current, which improves the sensitivity compared with other approaches, which should facilitate better integration into a sensory device.

Methods

The experiments focussed on the use of a nominally undoped silicon thin film transistor, the wafer was provided by Miplaza, Philips, The Netherlands. To create the electrodes a bottom-up technique has been used to fabricate the electrodes. A layer of 100 nm of aluminium (Puratronic 99.99 % purity) was deposited by evaporation on the silicon wafer using a Moorfields evaporator. The device consists of two rectangular electrodes of 1 mm×0.5 mm separated by a gap, which will form the sensor. The gap size was kept constant for all experiments and measured at 20 μm. After spin coating the resist (Shipley, S1813) the electrodes were patterned using photolithography. Subsequently the undeveloped resist was removed by washing in de-ionised water, followed by an aluminium etch step (aluminium etchant from Technic. Inc. used as received). The final steps were a de-ionised water wash to remove the etchant and etched aluminium, followed by an acetone wash for the residual resist removal.

All chemicals used for the functionalisation and the subsequent experiments were purchased from Merck and used as-received, unless stated otherwise. The aptamer probe was attached to the silicon channel using a silanisation method [18]. In brief this consisted of immersion in an ethanol solution containing 3% (v/v) APTES at 80 °C for 2 h. After washing the slides four times with ethanol and deionized water, the slides were dried under N2, and heated at 110 °C for 1 h. A cross-linker, glutaraldehyde was used to create the binding side for the aptamer. The samples were immersed in a glutaraldehyde/water solution containing 2% (v/v) glutaraldehyde at room temperature (25 ± 2 °C) for 1 h and then rinsed with deionized water, dried under N2. In the final step amine functionalised aptamers, Eurogentec, Belgium, were used. The aptamer solution (200 μL, 100 nM), incubated at 37°C for 2 h and then rinsed with PBS and deionized water to remove the excess aptamers, then dried under N2.

An aptamer that was previously reported to bind to the spike protein of the COVID-19 virus was used [17]. The sequence is 5’-CAGCACCGACCTTGTGCTTTGGGAGTGCTGGTCCAAGGGCGTTAATGGACA-3’.

The aptamer was functionalised with an amine terminal group at the 5’ end. Once the samples have been functionalised, the response to varying concentrations of the recombinant SARS-CoV-2 spike protein, S1 Subunit, Cambridge Bioscience, UK, was determined with serial dilutions of the spike protein in PBS. Prior to any characterisation the samples were exposed to a PBS solution without the protein. The samples were left in the solutions for 15 minutes and subsequently washed with PBS and blown dry with N2. A schematic representation of the functionalisation process is shown in Figure 3.

The electrical characterisation was done using a Hewlett-Packard 4155B semiconductor parameter analyzer. All electrical characterisation was performed in air on dried samples. The source-drain potential was varied between ±15 V with a step size of 15 mV and a settling time of zero. All measurements were performed by sweeping the potential from 0 to -15 V, a return sweep back to zero followed by a sweep to +15 V and subsequent return to 0 V. Due to the symmetry one of the electrodes was connected to ground and will be referred to as the source, while the potential of the other is varied, and designated the drain. All experiments were repeated on 4 different devices on the same sample, and across 6 different samples. While there were some variations in the current level, the shape of the IV-characteristics were the same and the effect of addition of the spike protein yielded a similar relative change.

Declarations

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

I.S. and H.v.Z conceptualisation, methodology, data analysis; T.F, S.L, I.S. and H.v.Z investigation, data acquisition; I.S. and H.v.Z. original draft; T.F, S.L, I.S. and H.v.Z review and editing

Competing Interests

The authors declare no competing interests.

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Figures

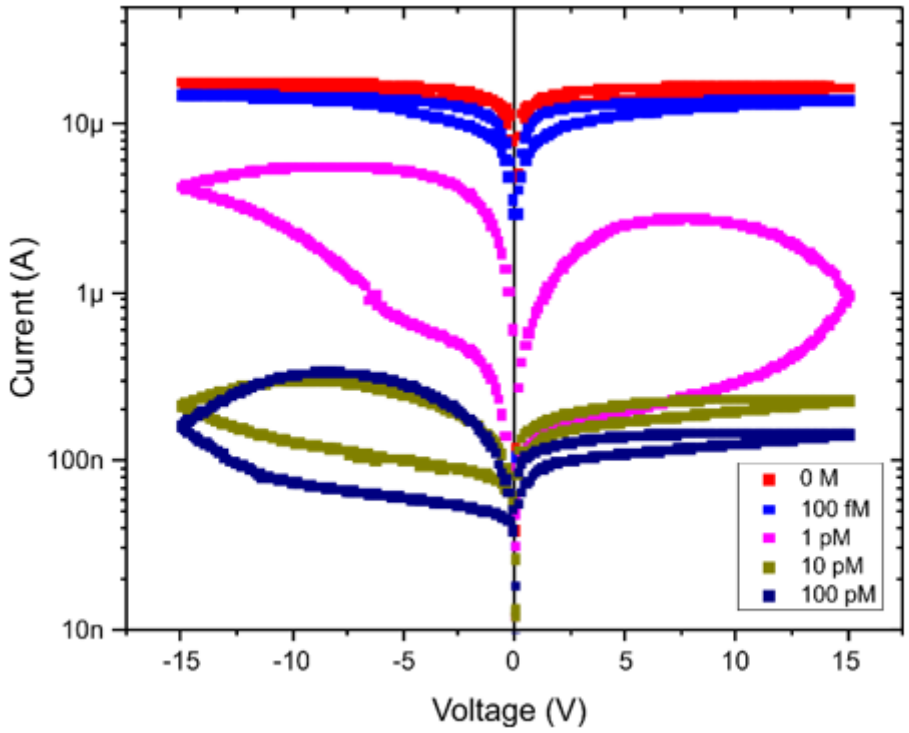

Figure 1

IV characteristics for different concentrations of the spike protein ranging from 0 M to 100 pM.

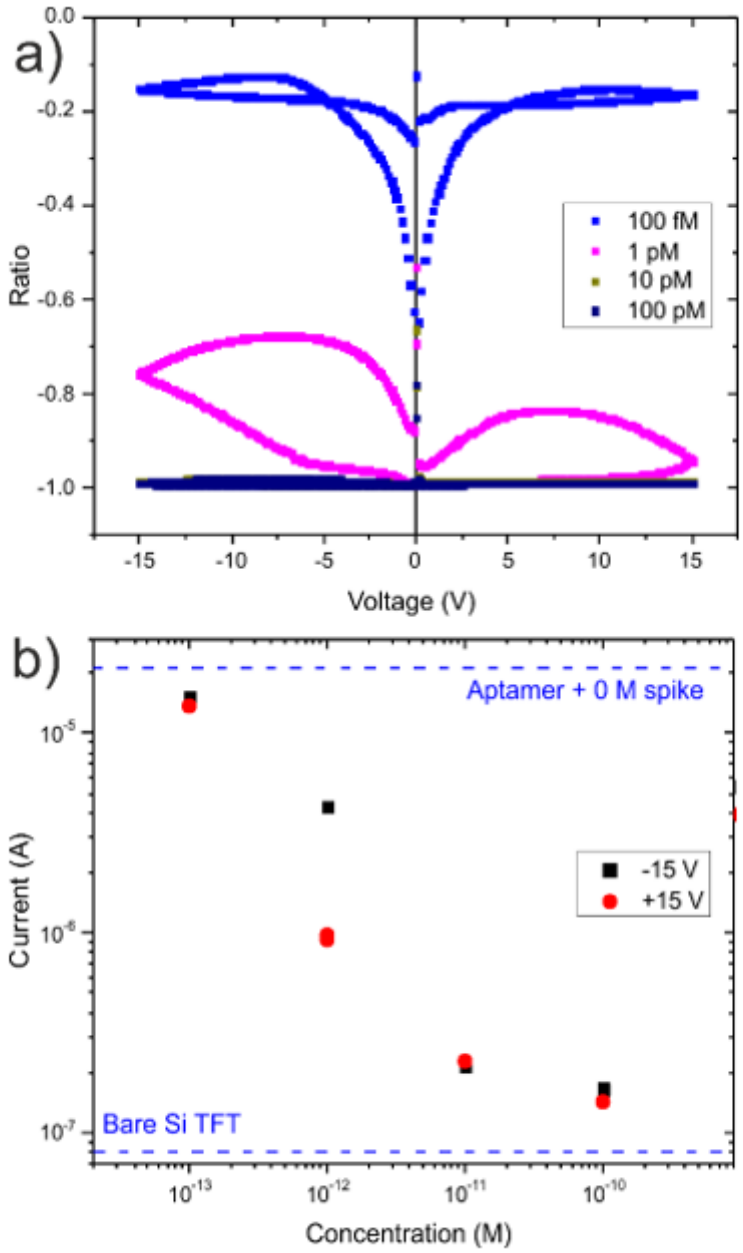

Figure 2

a. The Ratio, R over the whole voltage range for different concentrations of the spike protein; b) shows the current at ±15 V. Also indicated is the current at zero concentration and that of the unfunctionalised silicon TFT.

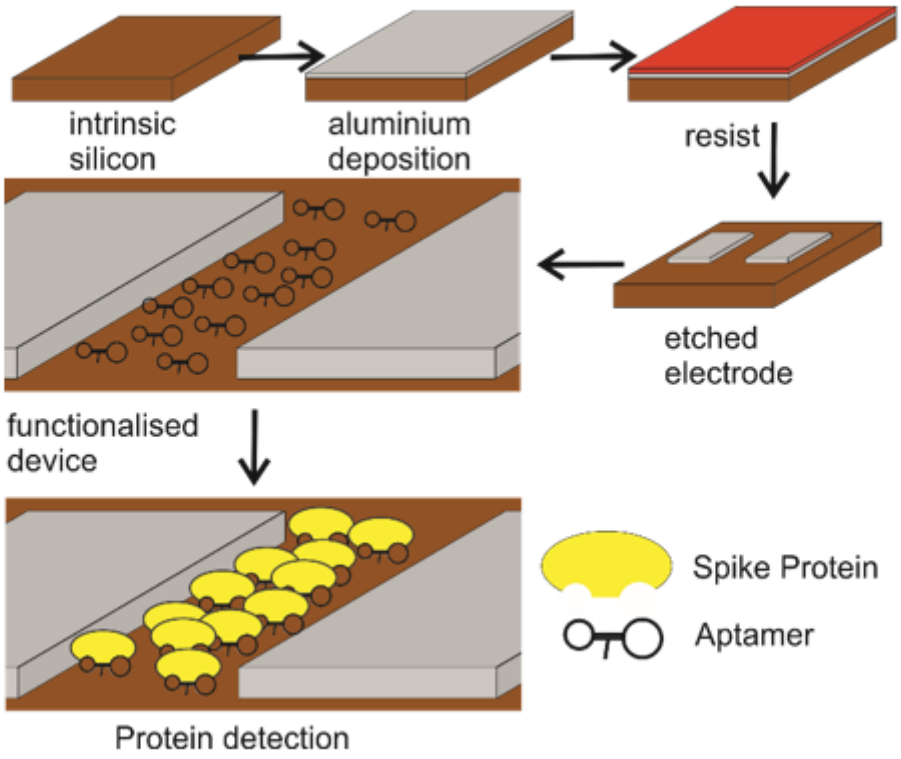

Figure 3

Schematic of the functionalisation process and subsequent detection of the spike protein.