Sybodies as novel bioreceptors toward field-effect transistor-based detection of SARS-CoV-2 antigens

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The SARS-CoV-2 pandemic has increased the demand for low-cost, portable and rapid biosensors, driving huge research efforts toward new nanomaterial-based approaches with high sensitivity. Many of them employ antibodies as bioreceptors, which have a costly development process requiring animal facilities. Recently, sybodies emerged as an alternative new class of synthetic binders/receptors with high antigen binding efficiency, improved chemical stability, and lower production costs via animal-free methods. Their smaller size is an important asset to consider in combination with ultrasensitive field-effect transistors (FETs) as transducers, which respond more intensely when the biorecognition occurs in close proximity to their surface. This work demonstrates the immobilization of sybodies against the spike protein of the virus on silicon surfaces, which are often the integral part of the semiconducting channel of FETs. Immobilized sybodies maintain the capability to capture antigens even at low concentrations in the femtomolar range, as observed by fluorescence microscopy. Finally, the first proof-of-concept of sybody-modified FET sensing is provided, using a nanoscopic silicon net as the sensitive area where the sybodies are immobilized. The future development of further sybodies against other biomarkers and their generalization in biosensors could be critical to decrease the cost of biodetection platforms in future pandemics.

1. Introduction
In the end of 2019, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was first identified and rapidly spread worldwide through human-to-human transmission. The World Health Organization classified this outbreak of coronavirus disease 2019 (COVID-19) as a global pandemic. According to WHO data, there have been a total of more than 600 million confirmed cases and more than 6 million deaths worldwide by the end of 2022.\[^1\]\(^\) Although several vaccines have been successfully developed in multiple countries, the virus continues to spread at a faster rate with the emergence of virus variants and inadequate vaccination rates.\[^2,3\]\(^\) Therefore, early diagnosis in the beginning of the disease can be crucial for outbreak control.\[^4\]\(^\) Currently, reverse transcription polymerase chain reaction (RT-PCR)-based viral RNA detection is considered the gold standard method for the diagnosis of COVID-19. However, RT-PCR methods are too expensive to use and usually require pooled samples to be performed in specialized laboratories, such that testing can only be selectively performed on high-risk individuals at the beginning of the outbreak.\[^5\]\(^\) An additional
consequence of needing sample delivery is the delay in the results, which is undesired in pandemic situations. Therefore, faster, more efficient and inexpensive assays have been introduced into the public. Rapid colloidal gold-based test kits were soon commercialized, but due to the low viral load of the virus in the early and late stages of infection, the detection rate of positive cases by rapid test kits was found to be low and therefore only could be used for primary screening.\textsuperscript{[6,7]} With the increasing demand for low-cost, portable and rapid biosensors, devices using nanomaterials (e.g., carbon nanotubes, graphene, metal oxide nanowires, and silicon nanowires) in combination with conventional metal oxide field-effect transistors (FETs) have emerged.\textsuperscript{[8–11]} Recently, field-effect transistors (FETs) have shown to be useful for the ultrasensitive diagnostics of cases like the SARS-CoV-2.\textsuperscript{[12]} After silicon nanowire-based FET sensing was first described by Lieber et al. in 2001,\textsuperscript{[13]} nanowire FETs have been also used for ultra-sensitive and selective detection of a wide range of biochemical analytes due to their miniaturization and high sensitivity.\textsuperscript{[14,15]} These have been applied to the sensing of DNA and RNA hybridization, the sensing of viruses and even the detection of individual viruses, multiplexed sensing using antibodies as receptors, saliva-based detection of human pressure levels and detection of cellular potentials by kinked nanowires.\textsuperscript{[16–20]} Special nanowire network geometries in the form of honeycomb were demonstrated by our team as an interesting case with enhanced area for interaction with the analyte.\textsuperscript{[19]} However, the Debye length limits the detection capability of such biosensors. While this distance is 0.7 nm for physiological ionic strength, a 10-fold dilution of the sample will result in a range of influence of 2.4 nm, while a 100-fold dilution will extend it to 7.4 nm.\textsuperscript{[21]} It can be noted that it is difficult to obtain an effective signal in 0.01 x PBS using antibodies (typical dimensions of IgG are approximately 14.5 nm x 8.5 nm x 4.0 nm) as receptors for biological recognition.\textsuperscript{[22]} Thus, it is evident that reducing the receptor size plays a critical role in improving the sensitivity of the FET-based biosensor. To overcome such limitations, smaller receptors have been applied, such as aptamers and nanobodies.\textsuperscript{[19,23]} With 12–15 kDa (~4.2 nm in length) for the nanobodies, the concentration of PBS solution should still not exceed 0.01x dilution. In contrast to nanobodies, which are split and isolated from natural antibodies, synthetic receptors like aptamers exhibit important advantages such as chemical stability and lower production costs, based on animal-free selection methods.\textsuperscript{[24]} However, nanobodies can also find their synthetic counterparts, also known as sybodies.\textsuperscript{[25,26]} Synthetic nanobodies emerged as a convenient way to generate receptors/binders against antigens, showing similar advantages to aptamers including rapid development and low production cost using prokaryotic expression systems.\textsuperscript{[27]} Being equivalent to nanobodies,
sybodies can be stored for several months at 4 °C and even longer at -20 °C with intact antigen binding capability. Incubation at 37 °C for several weeks has also shown good tolerance.\textsuperscript{[28]} It has even been shown that nanobodies can remain stable at temperatures up to 90 °C and higher, which is of great importance for the production, cleaning and storage for biosensing applications.\textsuperscript{[29]} Compared to isolating fragments from natural antibodies as nanobodies, the binders selection of synthetic nanobodies can be done more rapidly and at a relatively low cost.\textsuperscript{[30]}

The receptor-binding domain (RBD) of the spike protein of the SARS-CoV-2 has not been an exception in the search of sybodies, with a recent demonstration of a high affinity amino acid sequence with neutralizing activity through binding to the antigen.\textsuperscript{[27,30–32]} While such development had the aim of developing a novel therapeutic agent, the application of sybodies in the biosensors field is certainly a possibility as well. In order to use it as the biological recognition element of a biosensor, during the process of development of such biosensor it is of high importance to determine the immobilization method that will retain its extraordinary binding efficiency. The functionality of the anti-SARS-CoV-2 sybody after binding a surface for its application as bioreceptor immobilized on a transducer still remains an open question.

In this work, we label the receptors and the antigens with fluorescent tags and we follow a micro-contact printing approach to proof the successful immobilization of and posterior antigen binding capability of sybodies against the RBD of the spike protein of SARS-CoV-2. We perform the immobilization on silicon as a common semiconducting material found in FETs, including those based on nanostructures for an enhanced sensitivity, i.e. silicon nanowire FETs. We find fluorescence as the evidence signal of the biorecognition process in the pg/mL concentration range. Then, we provide the first proof-of-concept of Bio-FET sensing of the antigen using FETs with a silicon nanonet as semiconducting channel, modified with the sybodies (Figure 1), providing a comparison to the performance of antibody-modified FETs.
Figure 1. Conceptualization of SARS-CoV-2 detection with sybodies and antibodies with silicon nanonet-based field-effect transistors. (a) Depiction of the nanonet area of a FET with a sybody and an antibody against the spike protein of the virus. The protein falls inside the Debye length when it is recognized by the sybody, in contrast to the case of the antibody, due to its larger structure. (b) Optical microscopy image of the FET. The silicon nanonet is fabricated in the marked blue area between source and drain electrodes. (c) Scanning electron microscopy image with a magnification of the nanonet area.

2. Results

For immobilization of receptors on a silicon-based FET surface, and silicon in general, the substrate needs to be modified with an appropriate functional layer. These modifications are done to activate the surface by introducing different tail groups. Generally, silicon has an oxide layer around it, thus subsequent chemical modifications can be performed on this native (or thermal) oxide layer. Used for decades, the amino terminated silane 3-aminopropyltriethoxysilane (APTES) is one of the most popular silane in research. However, for receptors such as antibodies or sybodies, the surface with amino groups after functionalization with APTES is still not directly available to the receptor, but requires the use of a coupling agent such as the carbodiimide chemistry to activate carboxyl groups on the receptor molecule. After functionalization, the remaining functional groups that are not fully reacted are to be blocked. For this reason, we base our immobilization strategy on our previously reported protocol based on TESP (3-triethoxysilylpropyl succinic anhydride), which provides an anchoring point in the form of a succinic anhydride that does not require activation steps or further passivation of unreacted groups. Such anhydride groups react
(easily) with amino groups to form amide bonds in ring opening reactions. The surface modification steps are shown in Figure 2a. Using such a method it is possible to immobilize the receptor with the amino group on the surface. When this step is performed in a liquid environment, the hydrolysis of the unreacted anhydride group produces carboxyl groups that will prevent nonspecific binding to other biomolecules also containing amino groups. Therefore, after receptor immobilization using this method, the binding site passivation step can be omitted, thus simplifying the surface functionalization step.

Briefly, the silanization process using TESPSA is carried out in a desiccator heated by an infrared lamp (Figure 2b), where the external heat source evaporates TESPSA and brings it into contact with the silicon dioxide surface to initiate the silanization reaction. This step allows the immobilization of silanes with a succinic anhydride functional group on the oxide surface. The silanization was done on diamond pencil-cutted silicon wafer pieces as well as directly on the silicon nanonet-FETs fabricated via electron-beam lithography (EBL).
Figure 2. Characterization of silicon surface silanization and receptor immobilization. (a) Schematics of the functionalization, including silanization with TESPSA and immobilization of sybody. The insets show the contact angle of each step. (b) Setup for the silanization process.
Characterization and measurements on planar surfaces

The modification of silicon surfaces was first characterized by contact angle measurements. By measuring the contact angle between water and silicon wafer samples after surface functionalization at different steps, it is possible to verify whether the material layer is successfully modified on the surface at each step. Contact angle measurements were performed on silicon wafer samples with 500 nm oxide thickness, in static mode, after depositing 1.5 µL DI water drops. The inset photographs of the water droplets in Figure 2a show the experimental results. The wafer with such a thick oxide layer is very hydrophilic (contact angle <5°), allowing a clearer distinction between the surface with and without TESPSA. The silanization increased the angle to 53°, similar to that obtained previously.[36] Immobilizing the antibody resulted in a further significant increase in contact angle. The blocking step with bovine serum albumin (BSA) did not lead to any significant changes. Since the role of BSA here is to block the TESPSA molecules on the sample surface that have not successfully bound to the antibody, if the antibody and TESPSA react sufficiently and there are no remaining free binding sites on the surface, BSA blocking will not make changes to the surface properties. Moreover, even if some of the BSA remains on the surface, both the antibody and BSA molecules as protein structures may have similar effects on the surface properties and therefore the measurements may be similar, making it impossible to distinguish them by this method. Thus far, vacuum incubation of TESPSA on the silica surface and stable binding of the TESPSA modified layer to the antibody were successfully verified using the static contact angle measurement method.

Next, to allow for comparative observation, we employed fluorescence microscopy in order to observe the distribution of proteins tagged with fluorescent labels on flat silicon dioxide samples. For this, we printed the protein layers on TESPSA-modified surfaces via µ-contact printing, i.e. with the aid of a polymer stamp fabricated by soft lithography (Figure 2c).[37] The fabricated polydimethylsiloxane (PDMS) stamps had a parallel strip structure with 30 µm width and 70 µm spacing (Figure 2d). Commercially available fluorescent anti-human IgG antibodies were printed as model antibodies on silanized surfaces. The pattern was clearly
visible, with fluorescent bright lines and a high contrast compared to the dark background (Figure 2e). The measured intensity was found to be almost double than that obtained on non-silanized surfaces (Figure 2f), as quantified by measuring the pixel intensity (Figures 2g and 2h). The non-silanized surfaces received no blocking step, and therefore we attribute the weaker signal to the non-specific adsorption of antibodies on the oxide surface. Then, we repeated the test with fluorescent SARS-CoV-2 antibodies and sybodies. The antibodies were already commercially available with the fluorescent tag. The non-fluorescent sybodies were synthesized following a previously reported method (see experimental section), generating a high affinity sequence selected from a library
(QVQLVESGGGLVQAGGSLRLSCAASGFPVESENMHWYRQAPGKEREWVAAIYSTG
GWTLYADSVKGRFTISRDNKNTVYLQMNSLKPEDTAVYYCAVQVGYWGQGQT
QVTVS), and named after Sybody 23 (Sb23). We labeled them prior to the printing using a commercial conjugation kit (see experimental section) in order to verify their binding to TESPASA. In both cases, the printing resulted in strong fluorescent patterns on silanized surfaces (Figure 3), comparable to those obtained previously with anti-human IgG, indicating that effective binding between TESPASA and bioreceptors against the virus occurred. The patterns obtained with the sybodies were slightly weaker than those from the antibodies. We attribute this to the fact that the labeling was done in the laboratory with the conjugation kit, which may lead to a lower labeling yield compared to the commercial antibodies. However, the results provide sufficient evidence that the TESPASA functionalized surface can establish a stable connection with the sybodies.
Figure 4. Fluorescence microscopy results of SARS-CoV-2 antibodies (a,c) and nanobodies (b,d) with fluorescent labeling on TESPMA-functionalized surfaces.

As a last microscopy test, we confirmed the binding of the antigen and the receptors. Non-fluorescent antibodies and sybodies were first printed via μ-contact printing on TESPMA-functionalized oxide surfaces. Then, fluorescent antigens were incubated on the whole surface, expecting to have a biorecognition only on the patterned lines (Figure 5a). It is worthy to mention that the antigens used were originally non-fluorescent, needing to go through a labeling step as well using the conjugation kit. In the case of the antibody-modified surfaces, the pattern formed by the antigen biorecognition was already visible after 15 min incubation of 1 pg mL⁻¹ concentrations, with a further intensity increase when this was increased 100-fold (100 pg mL⁻¹) (Figure 5b).
Figure 5. Fluorescence microscopy results of SARS-CoV-2 antigen detection on surface with different chemistry. (a) Depiction of the followed methodology by μ-contact printing. A pattern of non-fluorescence receptors is introduced, followed by incubation of fluorescent antigens on the entire surface. The antigens only remain on the patterned area, which can then be visualized by microscopy. (b) Antigen detection on antibody-modified surface. (c) Antigen detection on sybody-modified surface (left) and non-specific anti-human IgG surface (right). On the latter, no binding occurs, and therefore no pattern is observed.

The pattern was visible as well after 15 min incubation of 1 pg mL⁻¹ antigen on sybody-modified surfaces (Figure 5c, left images). Despite the binding being not as efficient as in the case of using antibodies as receptors, the results show that the same concentration levels can be visualized. This cannot be attributed to non-specific binding, since the incubation was done on the entire surface. A lower labeling yield or conformational changes upon labeling due to the small peptide size cannot be discarded as well. A control experiment with anti-Human IgG antibodies instead of the sybodies as receptors confirmed that the RBD antigen could be captured only with when the appropriate receptors were present (Figure 5c, right images).
FET measurements

The bioreceptors against SARS-CoV-2 were immobilized on the oxide surface of the silicon nanonet of the FETs following the same protocol characterized before using TESPSA as silanizing agent. Then, they were incubated with 1 ng mL\(^{-1}\) and 10 ng mL\(^{-1}\) antigen solutions in order to compare the changes in the transfer characteristics. The incubation was done in PBS (pH 7.4) to simulate the biochemical environment similar to serum, in which proteins such as antigens and antibodies can be solubilized and maintain a more stable form and properties. In order to achieve immune binding of antigens and antibodies, positive ions in the solution are needed as intermediates to attract antigens and antibodies using van der Waals forces and thus bind. However, to enhance the effect of surface charge on the FET signal change, the measurements were performed in DI water, increasing the Debye length. The electronic signature of FETs could indeed be used as supporting characterization step of the nanonet functionalization itself, by comparing the transfer characteristics after the introduction of the receptors (Figure 6a). A blocked FET with only BSA but no receptors was used as unreactive control device. In all examples, a significant rightward shift of the overall curve can be observed after the functionalization takes place. Considering the low isoelectric point of the BSA (ca. 4.5) and its consequent negative charge at the pH value of the water used for the measurements (pH 8), this rightwards shift is explained. A similar shift is observed for both sybody and antibody modification. With the theoretical isoelectric point of 3.9 and negative net charge of \(z = -2.31\) of the RBD sequence (cngvefgncyflqsgfqp) at pH 8, the target molecules inhibit the formation of conducting channels in our n-type FETs. By measuring the current at the subthreshold regime (gate voltage \(V_g = 0.75\) V), both sybody- and antibody-modified FETs showed a current reduction of ca 40% for a set of three FETs each, after the first antigen incubation (1 ng mL\(^{-1}\)). Successive incubation with a higher concentration (i.e. 10 ng mL\(^{-1}\)) did not result in any changes, probably due to sensor saturation. It is known from previous publications that FETs with such nanonet architecture provide a response in the femtomolar range,\[14\] and in consequence the here tested levels (picomolar) quickly saturate the receptor layer. Hence, the sybodies maintain their biorecognition capability providing the FETs with a highly sensitive response toward SARS-CoV-2 antigens. Although the normalized response of the biosensors is found to be similar either with sybodies or antibodies, we attribute this to the use of DI water as measurement medium, where the Debye length can be as long as 100 nm.\[38\] However, the absence of ions can lead to negative effects in the stability of bioreceptors, therefore measuring in diluted...
buffer can be preferable, where the Debye length can be considerably shorter. In that case, we predict an improvement of the results obtained using sybodies due to their smaller size.

Figure 6. Field-effect transistor characterization of receptor and antigen binding. (a) Transfer characteristics of FETs before and after sybody or antibody binding. A rightward shift is observed in both cases. The inset shows the same effect for the surface blocking with BSA instead of receptor immobilization. (b) Bar graph showing the evolution of the FET current at the subthreshold regime with antigen biorecognition. The current drops significantly for sybody-modified FETs with the smallest tested concentration due to the rightwards shift of the transfer characteristics. The drop is not as strong in antibody-modified FETs. No significant changes are observed in FETs with BSA instead of receptors (blocked surface). The inset shows the shift in the transfer curve for a sybody-modified FET after 1 ng mL⁻¹ antigen incubation.

4. Conclusion
Nanomaterials, with their high surface area ratio and quantum size effect, possess physicochemical properties different from macroscopic scale, which lead in the development of biosensors. The selection of bioreceptors and the choice of bioreceptor immobilization method play an important role in biosensor performance. Based on their small size and high stability, sybodies hold great promise for highly sensitive biorecognition when integrated in silicon nanostructured FETs, by bringing the charged analyte layer in closer proximity to the semiconducting channel compared to antibodies. Their animal-free, synthetic nature offers an additional advantageous asset. The functionality of immobilized sybodies against SARS-CoV-2 on silicon-based supports and nanomaterials for FET-based sensing was still a pending issue that we have addressed demonstrating that the receptor maintains the biorecognition
capability. Detection via fluorescence was possible in low antigen concentrations down to 1 pg mL^{-1} (0.44 pM). We have provided as well the first preliminary results of biorecognition signal using sybody-modified silicon nanonet-FETs and compared to FETs with antibodies in the low picomolar levels, showing a similar performance. Further experiments in lower target concentrations and higher ionic strength using diluted buffer instead of DI water can help gaining further insights on the performance difference in shorter Debye lengths.

We foresee an important role in future FET biosensor platforms using sybodies as receptors for the detection of lower concentrations, providing ultrasensitivity and the advantage of the synthetic nature of the immobilized peptides. The development of new sybodies with high binding affinity for other antigens or biomarkers, together with the generalization of their use in other types of biosensors, could suppose a big step toward the decrease of production costs of biodetection platforms in future pandemic outbreaks where economic constricts typically occur.\[39\]

3. Experimental section

3.1 Expression and purification of Sybody 23 (Sb23)

Sb23 has been selected from a synthetic sybody library and targets the receptor binding domain (RBD) of SARS-Cov-2 with high affinity and is capable to neutralize the virus efficiently.\[30\] Sb23 expression and purification has been performed as described previously.\[30\] In short, Sb23 was expressed in TB medium after induction with 0.02% (w/v) L-Arabinose. After periplasmic extraction, SB23 was purified via metal affinity chromatography (IMAC) and desalted in PBS. The protein was concentrated, snap frozen in liquid nitrogen and stored at -80 °C until further use.

3.2 Si-nanonet FET fabrication

The FETs were fabricated following a highly reproducible electron-beam lithography process. The starting material was a silicon-on-insulator (SOI) wafer with a 100 nm top Si layer (p-type, 10 Ω·cm, (100)) and a 400 nm buried oxide layer. KrF photolithography and inductively coupled plasma reactive ion etching were used to form the active region. Afterwards, a photoresist mask was deposited on the channel region, followed by source and drain formation by As ion implantation with a dose concentration of 5 x 10^{15} cm^{-2}. Then, a rapid thermal annealing process was done at 1000 °C for 20 s. A gate insulator oxide layer was grown by using a pyro-oxidation furnace at 800 °C. The silver transmission lines and contact pads as well as the pseudo-gate electrode were fabricated using an I-line stepper and a lift-off
process. Next, an SU-8 layer was deposited on the whole surface as passivation layer, leaving only exposed to the environment the semiconducting channel, the contact pads and the pseudo-reference electrode regions. Finally, the pseudo-reference electrode was turned into a true reference electrode by an electrochemical reaction forming an Ag/Cl layer.

3.3 Surface modification and characterization

Surface modification

Four-inch silicon wafers with 500 nm top oxide were first divided into samples of approximately 10 mm x 20 mm, and the samples were cleaned by immersing in acetone, isopropanol, and water for 5 min each in an ultrasonic bath. After that, the samples were dried with N\textsubscript{2}.

In order to increase hydroxyl group content on the silica surface, the samples were treated with 100 W oxygen plasma for 10 seconds (Zepto Plasmacleaner, Diener electronic GmbH & Co KG, Germany). The plasma-treated sample was then placed in a medium-sized Petri dish with the treatment side up. An eppendorf cap containing 100 µL of TESPSA (abcr GmbH, Germany) was placed in the middle of the Petri dish. Two pieces of aluminum foil were folded over the edge of the Petri dish to prevent the Petri dish from being completely closed in vacuum. The petri dish was placed into the desiccator and closed tightly. The desiccator was connected to a vacuum pump and heated with an infrared lamp (Sanitas SIL 16, Sanitas, Spain) during four hours. Immediately after the vacuum incubation, the samples were transferred to glass petri dishes and incubated for 30 minutes at 120 °C. Next, receptor proteins (antibodies and sybodies) where immobilized on the surface of the samples. For planar samples that were analyzed by contact angle, the entire surface was modified by drop casting 50 µL of protein solution (0.1 mg mL\textsuperscript{-1} in PBS). The samples were incubated for 1.5 h in a humid atmosphere to avoid drying. The unreacted TESPSA molecules were blocked by an additional incubation step with 0.1% BSA. The samples were finally rinsed with PBS to remove loosely bound proteins. FETs were functionalized following the same procedure with exception of the ultrasonic bath in order to avoid nanowire damage.

Planar samples that were analyzed by fluorescence microscopy followed a different methodology after the silanization. The receptor proteins were patterned by µ-contact printing. For this, the first step was to fabricate a PDMS stamp from a mould. The mould consisted of an SU-8 structural layer etched by UV lithography. The PDMS negative copy was fabricated by soft lithography using a 10:1 ratio of elastomer and crosslinker (Sylgard 184 Silicone Elastomer Kit from Dow Corning, purchased from Distrelec Deutschland
GmbH, Germany). For the transfer of the proteins with the same pattern to the silicon substrates, a solution containing the molecules to be printed (so-called ink) was dropped onto the surface of the stamp, covering the entire structured area, and gently shaken to expel air bubbles. After 1 h, the ink was then gently blown away from the stamp surface using a nitrogen gun, until there were no visible droplets on the surface, followed by quickly pressing the PDMS stamp onto the target surface to ensure sufficient contact. The PDMS was left on the silicon substrate under 5 g of weight for 1.5 h. The surfaces were finally rinsed with PBS.

Characterization
Static contact angle: An OCA system (DataPhysics, Germany) was used to measure contact angle. We deposited 1.5 µL DI water drops and measured the angle formed between the droplet and the surface. Variations in this angle are determined by the chemical properties and the roughness of the surface. Having a constant roughness of the surface, the changes in the measured angle were a result of surface modification with silanes and proteins. The results were averaged for three droplets per sample.

Fluorescence microscopy: an inverted microscope Axiovert 200 (Zeiss, Germany) was used for the visualization of the fluorescent samples. Two steps were verified using fluorescence microscopy. On one hand, fluorescently labeled receptors were printed by µ-contact printing on silicon wafers with and without TESPSA to verify their covalent immobilization. On the other hand, fluorescence microscopy could also be used to verify the specific immune binding between receptors and antigens. For this, non-fluorescent receptors were patterned on TESPSA-modified surfaces via µ-contact printing. Then, 50 µL antigen were deposited covering the entire surface. After thorough rinsing with PBS, the antigens should remain only on the patterned area giving rise to the fluorescent parallel lines. The fluorescent labeling of the sybodies and antigens was done using a commercial conjugation kit (ab188285 – FITC Conjugation Kit (Fast) - Lightning-Link (Abcam, United Kingdom). Commercial fluorescent anti-IgG antibodies (FITC-labeled) were purchased from Sigma Aldrich (Germany). Fluorescent and non-fluorescent recombinant antibodies against the spike protein of SARS-CoV-2 were purchased from Abcam (United Kingdom). SARS-COV-2-Spike-RBD epitope (480-499) was purchased from Sigma Aldrich (Germany).

3.4 Silicon nanonet-FET biosensing
To analyze the characteristics and performance of the FETs, a probe station with micropositioners was used to connect the source, drain and gate electrodes to a source
measure unit (Keithley 2604B, Keithley Instruments GmbH, Germany). The measurements were done in DI water, adjusting the droplet volume to the size of the sensing area (0.5 µL). The transfer characteristics were acquired by sweeping the gate voltage while measuring the resulting source-to-drain current, at a constant source-to-drain voltage of 200 mV. For antigen detection, 0.5 µL droplets of the antigen solution (1 ng mL⁻¹ and 10 ng mL⁻¹) were incubated on the sensing surface for 15 min and then rinsed with PBS and DI water, carefully dried with nitrogen and followed by the deposition of a DI water droplet of the same volume. The transfer characteristics were acquired with such droplet on the FET surface.

Acknowledgements
This work was funded by the Sächsische AufbauBank project 100525920 and the Federal Ministry of Education and Research (BMBF, grant number 05K18YEA).

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