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# The Future of Nanotechnology-Driven Electrochemical and Electrical Point-of-Care Devices and Diagnostic Tests

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*Annual Review of Analytical Chemistry*  
The Future of  
Nanotechnology-Driven  
Electrochemical and Electrical  
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Diagnostic Tests

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### Keywords

electrical, electrochemical, point-of-care devices, diagnostics, biosensors, nanotechnology

### Abstract

Point-of-care (POC) devices have become rising stars in the biosensing field, aiming at prognosis and diagnosis of diseases with a positive impact on the patient but also on healthcare and social care systems. Putting the patient at the center of interest requires the implementation of noninvasive technologies for collecting biofluids and the development of wearable platforms with integrated artificial intelligence-based tools for improved analytical accuracy and wireless readout technologies. Many electrical and electrochemical transducer technologies have been proposed for POC-based sensing, but several necessitate further development before being widely deployable. This review focuses on recent innovations in electrochemical and electrical biosensors and their growth opportunities for nanotechnology-driven multidisciplinary approaches. With a focus on analytical aspects to pave the way for future electrical/electrochemical diagnostics tests, current limitations and drawbacks as well as directions for future developments are highlighted.

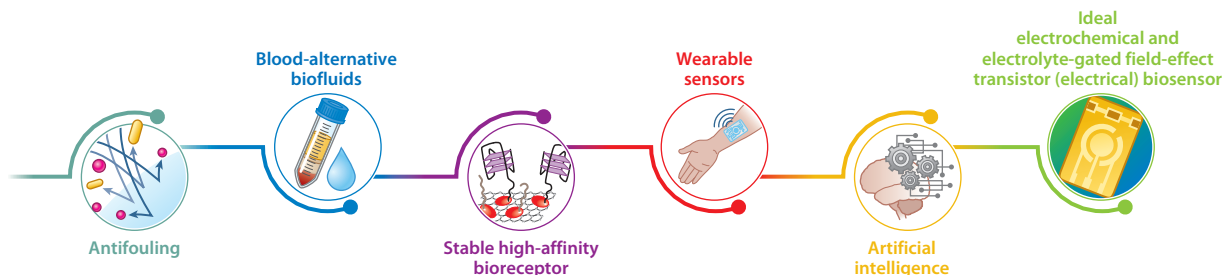
## INTRODUCTION

Increased life expectancy worldwide, changing health needs, and the growing awareness and public expectation of the health system are raising the bar for diagnostic systems to produce better health outcomes with advanced and patient centered diagnostic platforms. The hope is that technological advances in electrical and electrochemical biosensors will lead to the next generation of diagnostic tools. Although everybody likely agrees that electrochemical and electrical biosensors are promising tools for clinical diagnostics, after years of intensive research by many laboratories, they still face technological challenges limiting their clinical implementation and fulfillment of global healthcare needs. As sensors are complex systems where each step must be carefully designed, bottlenecks are encountered at different stages. Some of the challenges facing the development of biosensors include improvement of both diagnostic performance and capacity for multiple analyte sensing; the production of point-of-care (POC) devices on an industrial scale or the production of wearable technologies with high reproducibility; biomarker detection with limited surface fouling; and the possibility of having selective bioreceptors on hand quickly in the case of a new pandemic. Several strategies involving nanomaterials and nanotechnological know-how, advances in structural biology and bioreceptor design, and novel findings in a series of clinical studies have recently allowed researchers to overcome some of the pending hurdles and initiate the consideration and implementation of alternative sensing approaches. Some of these strategies were quickly exploited during the SARS-CoV-2 pandemic, such as the focus on less-invasive biofluid sampling for virus analysis and replacing nasopharyngeal swab samples with saliva or exhaled breath and its condensate without compromising valuable information on the patient's health condition (1, 2). The importance of rapid and easy access diagnostics is not limited to virus infection. It also includes the early detection and treatment monitoring of high-mortality diseases, such as various cancers, cardiovascular disease, and bacterial infections and inflammation in hard-to-heal wounds. Wearable sensors in the form of intelligent electronic skin bandages or breathing masks with integrated sensors might be ideally suited for exploring the time profile of established biomarkers in biological samples that can be noninvasively collected (e.g., tears, sweat, saliva, breath) and providing hospital-to-home solutions for health monitoring at reduced costs. Taking the example of wound infections, which are more explicitly discussed later in this review, the traditional methods used to identify wound infections or inflammation through visual inspection and microbiological analysis of wound samples have serious limitations in determining infection status, particularly in early phases or in complex and hard-to-heal wounds (3). Early sensing of wound biomarkers in the wound bed would enable faster and appropriate intervention with enhanced wound healing without the requirement for systemic antibiotic therapy.

The development of biosensors has been driven by their great potential to overcome barriers to traditional medical diagnosis for health monitoring and emergency diagnosis; the reader is referred to other reviews on this vast topic (4–6). This review provides some insights into the great potential of electrochemical and electrical biosensors and discusses how technological advancements are adapted to solving future challenges for their use as POC sensing devices (**Figure 1**). We do not provide a comprehensive review of the literature, but rather a collection of important advancements focused on electrochemical diagnostic platforms and electrical platforms in the form of electrolyte-gated field-effect transistors (FETs) that we consider technologically relevant.

## DIAGNOSTIC PLATFORMS USING ELECTROCHEMICAL AND ELECTROLYTE-GATED FIELD-EFFECT TRANSISTORS AS TRANSDUCERS

Since the introduction of the enzyme-linked immunosorbent assay (ELISA) in 1971 (7), its application in medical laboratories for the quantification of protein biomarkers has steadily grown.



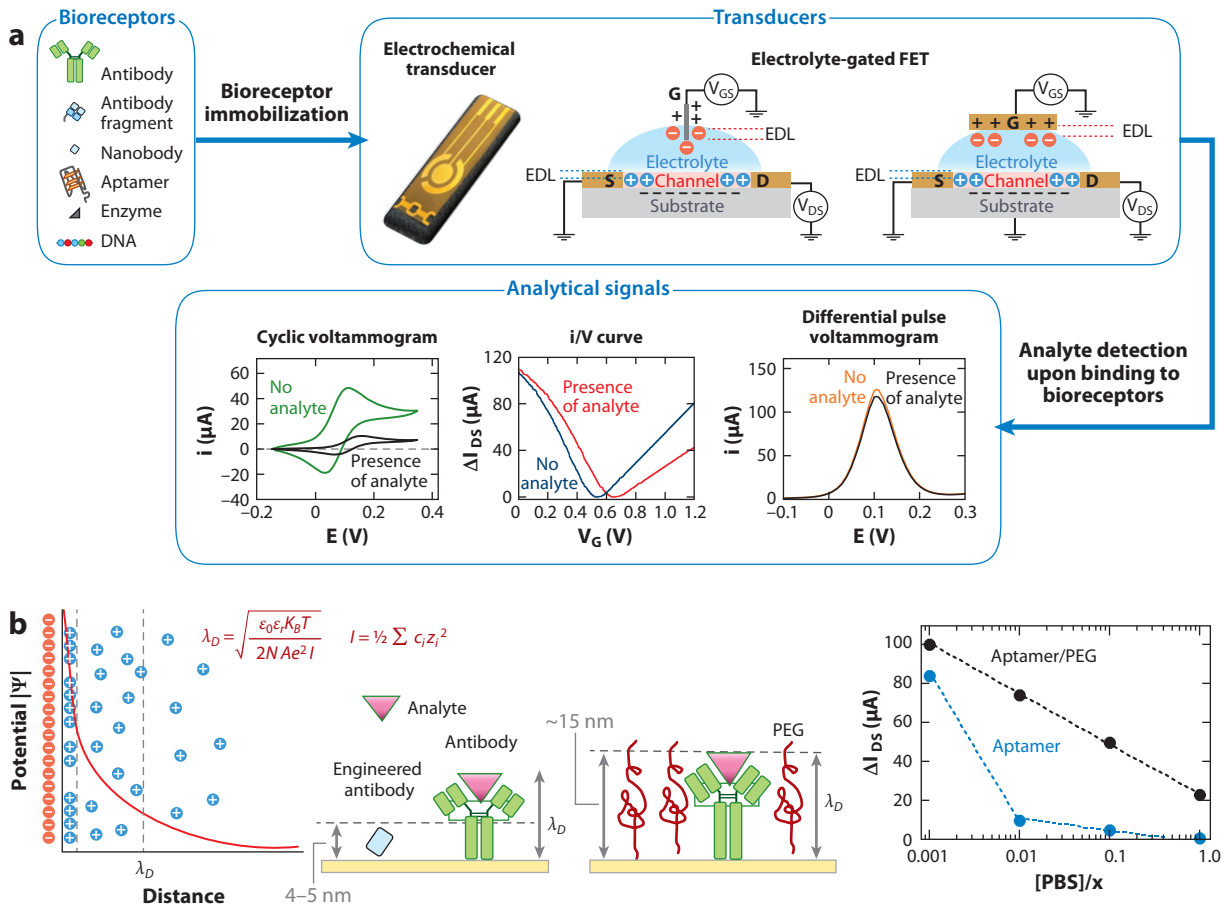
**Figure 1**

Technology-driven breakthroughs to build a future with more ideal electrochemical and electrolyte-gated field-effect transistor (electrical) diagnostic approaches. These include antifouling strategies using nanomaterials, the use of alternative biofluids such as sweat or breath for sensing, novel high-affinity bioreceptors in the form of aptamers and engineered antibodies, wearable sensors, and data analysis via artificial intelligence.

Despite its high sensitivity, accuracy, and reliability, ELISA requires rather expensive reagents and equipment, labor-intensive and time-consuming pretreatments, and skilled personnel. These features impact the development of inexpensive, miniaturized, and easy-to-use devices into POC devices. The major technological challenges of POC-based molecular diagnostics involves the development of methods to detect biomarkers with high selectivity and reliability comparable to those of ELISA. Because essential components of a POC device are transducers that convert a biological recognition event into something quantifiable and processable, biosensor development has become increasingly important. Biosensors typically comprise an analyte-specific bioreceptor that is immobilized on a transducer, producing a signal change upon the binding event.

Electrochemical sensors (**Figure 2a**) provide low-cost solutions for the detection of various analytes in different solution environments (8, 9). As exemplified by the glucose sensor, electrochemical biosensors do not suffer the drawback of highly complex sensor setup and provide an attractive means to analyze the content of a biological sample quickly and with high sensitivity and selectivity. Other inherent advantages of electrochemical biosensors are their robustness, easy miniaturization, excellent detection limits, including the use of small analyte volumes, and the ability to be utilized in turbid biofluids. Advances in electronic instrumentation offer the advantage of miniaturization of electrochemical biosensors as handheld devices (10–12). The biggest ongoing challenges are obtaining low limits of detection (LODs), suppressing the nonspecific adsorption of interfering species, and maintaining reproducibility and stability of the sensor in complex matrices such as whole blood (**Figure 1**).

FET-based diagnosis also faces unaddressed challenges, particularly for sensing in complex media such as whole blood. Boosting the performance and clinical use of transistor-based POC devices requires considerations of the implementation of alternative bioreceptors and the use of nanomaterials to boost the performance of FETs. Electrolyte-gated FETs (**Figure 2**) are like electrochemical sensor three-terminal devices (13) and are a preferred FET configuration for biomarkers present in biological fluids, as they allow for sensing in the fluid sample directly without intermediate drying steps. In these devices, the conductivity of a semiconducting material (the sensing channel) connected to two electrodes (the source and the drain terminals) is modulated by a third electrode known as the gate. Numerous geometries are described in the literature (14), with the working principle of these FETs being the following: A voltage applied to the gate modifies the charge carrier density in the semiconductor channel between source and drain and modulates the drain-source current ( $I_{DS}$ ). This ionic–electronic modulation generates a large variation in channel conductivity that, in turn, manifests in a large modulation of the electronic source to drain current flowing through the transistor channel.



**Figure 2**

Electrochemical and electrolyte-gated FET-based transducer. (a) Design considerations of biosensors based on the linking of a bioreceptor onto the surface of the sensor (transducer), the working electrode in electrochemical sensors, and the semiconductor channel in the FET. This is followed by the detection of a change in electrochemical (e.g., cyclic voltammograms, differential pulse voltammograms) or electrical [e.g., transfer curves ( $I_{DS}$  versus  $V_G$ )] signals upon analyte/bioreceptor interaction. (b) Correlation of bioreceptor size and effect of high-molecular-mass PEG on the  $\lambda_D$  in high-ionic-strength media showing an example of the change of  $I_{DS}$  with an increased PBS concentration of an aptamer/PEG-modified, graphene-based FET compared to an aptamer sensor. Panel *b* adapted with permission from Reference 63; copyright 2022 Elsevier. Abbreviations: EDL, electric double layer; FET, field-effect transistor;  $I_{DS}$ , drain-source current;  $\lambda_D$ , Debye length; PBS, phosphate-buffered saline; PEG, poly(ethylene glycol);  $V_{DS}$ , drain-source voltage;  $V_G$ , gate voltage;  $V_{GS}$ , gate-source voltage.

For electrolyte-gated FETs, the decay constant or Debye length  $\lambda_D$ , defined as the distance where a net charge is screened to  $1/e$  of its maximum value by mobile ions in the medium, plays a key role in the sensing performance (15–17) (**Figure 2b**). According to the Debye-Hückel model, charged molecules in solution are screened by mobile counter-ions such that their electrical potential is exponentially dampened. Distance  $\lambda_D$  is the Debye length given by

$$\lambda_D(\text{nm}) = \sqrt{\frac{\epsilon T k_B}{2N_A I e^2}} \quad [\text{at room temperature } \lambda_D = 0.304/\sqrt{I}], \quad 1.$$

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with  $k_B$  being the Boltzmann constant,  $T$  the temperature,  $N_A$  Avogadro's number,  $e$  the electron charge, and  $I$  the ionic strength of the solution. Charges located outside  $\lambda_D$  are considered out of range for electrostatic gating-based detection by a FET sensor. Under physiological condition ( $>150$  mM or  $1 \times$  PBS), this accounts for  $\lambda_D = 0.7$  nm and increases to 2.4 nm ( $0.1 \times$  PBS) and 7.4 nm ( $0.01 \times$  PBS) in lower ionic strength solutions. With the length of antibodies measuring approximately 10–15 nm, there is an intrinsic mismatch in dimensions between the bioreceptor and the charge screening. Alignment with the life sciences is possible through the use of antibody mimetics of small size (18) and/or the use of high-molecular-weight polyethylene glycol (PEG) layers, as demonstrated by Lieber and colleagues in 2015 (16) using the detection of prostate-specific antigen (PSA) in 0.1 M phosphate-buffered saline (PBS) as a model. Indeed, next to the well-organized charged layer on top of the semiconductor channel and guiding the Debye length in the presence of a PEG layer, any charges or dipoles leading to a charge within that immobilized ion permeable layer require the extra accumulation of a counter-ion within the layer to maintain charge neutrality. This difference in the concentration of ions between the bulk solution and immobilized protein layer creates a Donnan potential extending beyond the Debye length (19). The presence of the Donnan potential was experimentally revealed by Gokturk et al. (19); this provides an increased understanding of how to design biological FET sensors and demonstrates the importance of overcoming antifouling issues next to the Debye length screening effects simultaneously. These advancements together with the compactness and scalability of these novel tools, their small size, and limited requirements for power of operation are highly desirable for designing rapid, label-free, and wearable diagnostic POC platforms. Their commercial availability and high-quality production on a large scale render them one of the most preferred sensing platforms.

## **ANALYSIS IN COMPLEX BIOLOGICAL FLUIDS SUCH AS WHOLE BLOOD**

Detection of disease biomarkers from whole blood is vital in disease prevention and management. However, because blood is a complex biofluid and biomarker, sensing in whole blood remains challenging. The main component is plasma composed of water (91%) along with proteins (hormones, antibodies), lipids, ions, nutrients, dissolved gases, and other metabolites. Human serum albumin accounts for 60% of the total plasma proteins (approximately  $35\text{--}50$  mg mL<sup>-1</sup>), followed by immunoglobulin G ( $6\text{--}16$  mg mL<sup>-1</sup>) and fibrinogen ( $2$  mg mL<sup>-1</sup>). Blood-based sensing can be achieved through preanalytical steps using magnetic separation and in-line microfabricated devices that operate upstream of the nanosensors (20). Surface chemistry advancements using either an antifouling peptide-modified interface (21, 22) or three-dimensional (3D) nanomaterials (23–25) have contributed significantly to the field.

### **Magnetic Separation and Fluid-Based Preanalytical Assays**

Magnetic separation assays, combining magnetic enrichment with electrochemical readout, are one of the first attempts at biomarker analysis in whole blood (26).

Microfluidic paper-based analytical devices ( $\mu$ PADs) (27) utilizing capillary action-based lateral and/or vertical fluid flow in paper-like materials to perform (bio)chemical reactions have experienced unprecedented success owing to their many advantages, such as being low-cost, eco-friendly, portable, and high-throughput manufacturing devices (28).  $\mu$ PADs are excellent tools for performing blood tests due to their ability to manipulate fluids without needing external pumps and to conduct rapid and low-cost diagnostics and analyses in a nonlaboratory environment. To detect target analytes, paper-based devices incorporate various techniques of bioanalysis, with electrochemical detection approaches representing useful analytical systems with wide applicability (29–31).

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Thread-based microfluidic devices have recently been shown to be capable of spontaneously transporting fluids due to capillary action without the need for external pumps or sophisticated microfabrication apparatus (32). A thread-based microfluidic combined with electrochemical readout for selected model analytes (dopamine, hydrogen peroxide, and *tert*-butylhydroquinone) in saliva was reported (33) based on the insertion of cotton thread achieving a constant and continuous flow rate ( $0.38 \mu\text{L s}^{-1}$ ).

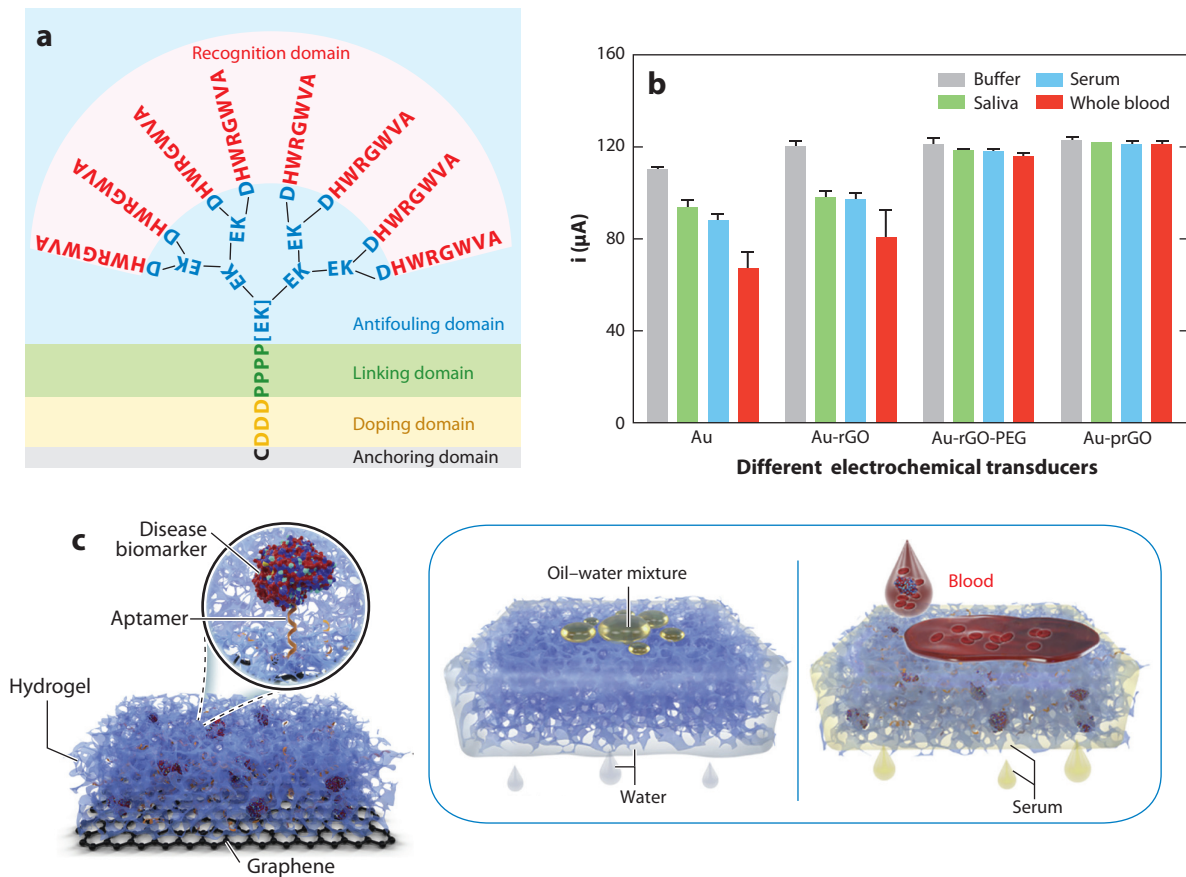
In addition to  $\mu$ PADs and thread-based microfluidics, lateral flow assays (LFAs), also known as immunochromatographic assays, provide a simple platform for POC diagnostics. Two of their most famous examples are the pregnancy test and SARS-CoV-2 antigen test. Although their sensing performance has been constrained by the optical readout of nanoparticle agglomeration, which typically allows only qualitative measurements, advancements made with cell phone camera readouts of LFAs have moved the analytical concept forward (34). An alternative strategy for obtaining true quantitative outputs is the integration of electrochemical transduction into LFA strips (35). However, printing metal electrodes on nitrocellulose membranes using commercial inks is challenging, due to the use of organic solvents in their formulations, which damage the delicate membrane. Instead of directly printing metal inks onto the nitrocellulose membrane, attempts were made to attach external electrodes to the detection area of the LFA (36). Güder and colleagues (37) have recently advanced this method by employing electrochemical LFAs using a near-field communication-enabled potentiostat and nitrocellulose-based metal electrodes.

### Surface Chemistry Considerations

Along with magnetic separation and fluid-based preanalytical sample preparation concepts, surface chemistry has long been of great interest for providing antifouling character to electrical and electrochemical sensors. As most electrical surfaces tend to be hydrophobic, they promote blood species adsorption possessing hydrophobic components. Because these hydrophobic interactions are entropically favorable in aqueous electrolytes and because water molecules are released from the solvation shell around hydrophobic compounds, fouling by a hydrophobic mechanism is typically irreversible in an aqueous electrolyte under mild conditions (38, 39). Many antifouling strategies consequently aim at reducing fouling by increasing the hydrophilicity of the electrode surface. The use of PEG and self-assembled monolayers carrying  $\text{CH}_3$  and  $\text{OH}$  terminal groups has been of great interest (40). Zwitterionic carboxybetaine-based polymer brushes showed efficient antifouling properties on gold electrodes against fibrinogen adsorption over wide pH and ionic strengths, although protein adsorption tends to increase at low ionic strength and low pH value (41).

Peptides based on alternating positively charged lysine (K) and negatively charged glutamic acid (E) groups have been considered as antifouling coatings based on the knowledge that EK-sequenced peptides possess zwitterionic characteristics and interact strongly with water molecules (21, 22). Luo and coworkers (22) pushed this technique further by constructing the YVEYHLCREKEKEKEKAKAKAKAK peptide that has four domains of interest (**Figure 3a**): The AKAKAKAK domain is applied for surface anchoring, as the positively charged sequence can adsorb on negatively charged interfaces such as electrodes modified with poly(3,4-ethylenedioxythiophene) (PEDOT) citrate films. To this was added an antifouling part (EKEKEKEK) and the recognition sequence YVEYHLC, specific to aminopeptidase N with nanomolar affinity. This sensor responds selectively to aminopeptidase N even in the presence of bovine serum albumin (BSA) ( $1 \text{ mg mL}^{-1}$ ) and HeLa cells ( $1.0 \times 10^4 \text{ cells mL}^{-1}$ ) (22).





**Figure 3**

Innovative strategies for diagnostics in whole blood. (a) Three-in-one peptide-based bioreceptor with surface anchoring, antifouling, and recognizing capabilities. Panel a adapted with permission from Reference 22; copyright 2020 American Chemical Society.

(b) Antifouling properties of different electrochemical transducers determined by the oxidation currents of ferrocenemethanol after exposure of electrodes for 24 h to different solutions. (c) Modified gFET with hydrogel-aptamer hybrid layer to achieve detection of disease biomarkers in whole blood without pretreatment of samples. Panel c adapted with permission from Reference 46 (CC BY-NC-ND 4.0). Abbreviations: gFET, graphene-modified field-effect transistor; PEG, poly(ethylene glycol); prGO, porous-reduced graphene oxide; rGO, reduced graphene oxide.

Recent developments in materials science have proposed novel approaches for inhibiting the occurrence of surface fouling processes. The use of porous electrodes as diffusion filters to alleviate fouling of proteins while allowing diffusive access to smaller analytes dates back to work by Patel et al. (42), which experimentally demonstrated that nanostructured gold electrodes have better antifouling properties than planar and macrostructured electrodes. The rationale behind this observation was that although proteins readily adsorb on planar gold electrodes, fouling of the pores is slower due to restricted mass transport of larger species through small pores. This approach has shown its limitation in DNA sensing, where the accessibility of target DNA was partly suppressed in nanoporous electrodes with small pore size, which are ideal for efficient biofouling (43). The antifouling potential of nanostructured electrodes forms the basis of the current approach proposed by Ingber and coworkers (25) to enable affinity-based electrochemical biosensing in complex biological fluids. Using a 3D porous matrix of cross-linked BSA and



glutaraldehyde supported by conductive gold nanowires, these electrode coatings preserved 88% of the original current signal even after 1 month of exposure to whole blood. Immobilizing interleukin 6 (IL-6)-specific antibodies allowed high-sensitivity sensing of IL-6.

The use of reduced graphene oxide (rGO) and its derivatives has added to the search for non-fouling interfaces in different ways (23, 45). Modification of a gold electrode by electrophoretic deposition of a porous rGO/polyethyleneimine (rGO/PEI) composite thin film (45) resulted in electrodes with excellent antifouling properties (**Figure 3b**) comparable to a 3D porous matrix of cross-linked BSA-glutaraldehyde supported by conductive gold nanowires (25). These results are in line with work by Ingber and coworkers who used nanomaterials as an antifouling electrode coating. Glutaraldehyde-based cross-linking of BSA in the presence of aminated rGO or pentaamine-modified graphene nanoflake has shown great potential as an antifouling electrode coating (23, 24).

In the case of an electrolyte-gated FET, Wang et al. (46) recently proposed the use of a 3D hydrogel-aptamer structure for the ultrasensitive and rapid screening of acute myocardial infarction in blood samples via its self-filtration ability (**Figure 3c**). The graphene-sensing channel was modified with a hydrogel composed of acrylamide and *bis*-acrylamide mixed with Acrydite-modified aptamers (H-AHL layer) via self-assembly. The super-hydrophilic wettability of the H-AHL layer with holes  $<5 \mu\text{m}$  enables the prevention of nonspecific adsorption of nontarget molecules such as lipids and hemicubes, but it allows proteins to pass with no influence from the biomarker diffusion rate to the sensing surface (46). Owing to this self-filtrating capability of the sensor interface, whole-blood analysis could be achieved, which moves this method a step further in aligning FET technology with diagnostic demands.

## BIORECEPTOR CONSIDERATIONS

The ability to selectively recognize the analyte of interest is fundamental to any biosensor. Considerations about the affinity of the bioreceptor as well as size, position of binding site, stability, and cost in most cases guide the choice of one bioreceptor over another. Enzymes, oligonucleotides, and antibodies are the most common bioreceptors for biosensors because they are commercially available. Peptides, aptamers, and antibody mimetics of smaller size and stability are currently at the forefront of investigation as bioreceptors for the goal of making sensors more robust and sensitive. The literature on these topics is vast, but because of its focus on LODs, it ignores other analytical performance parameters such as batch-to-batch viability, upscaling possibilities, and reproducibility and whether reaching better sensitivity has diagnostic merit. Avoiding this narrow focus will allow biosensors to more quickly obtain higher technological readiness levels and more readily reach the diagnostic market.

### Enzymes as Bioreceptors

The use of surface-immobilized enzymes as bioreceptors is one of the oldest and most popular approaches for biosensors to monitor small analytes such as glucose, lactate, and cholesterol. The use of glucose oxidase in glucose sensors is indeed one of the greatest examples of an electrochemical sensor with stunning commercialization success. The possibility of substituting the enzyme with enzyme-like activity materials has seen intensive research input (47–49). Attention has been paid to metal and metal oxide nanoparticles that are able to replace enzymes such as horseradish peroxidase (HRP) in sandwich-like assays (50, 51). Besides, by replacing the natural enzymes with enzyme mimetics as candidates, stabilization of enzymes such as glucose oxidase is made possible by embedding the catalytic system into synthesized polymers (52). Azzaroni's team (52) recently showed that electropolymerization of a layer of poly(3-amino-benzylamine-*co*-aniline) (PABA) on a FET provides suitable electrostatic charge and a non-denaturation environment for glucose

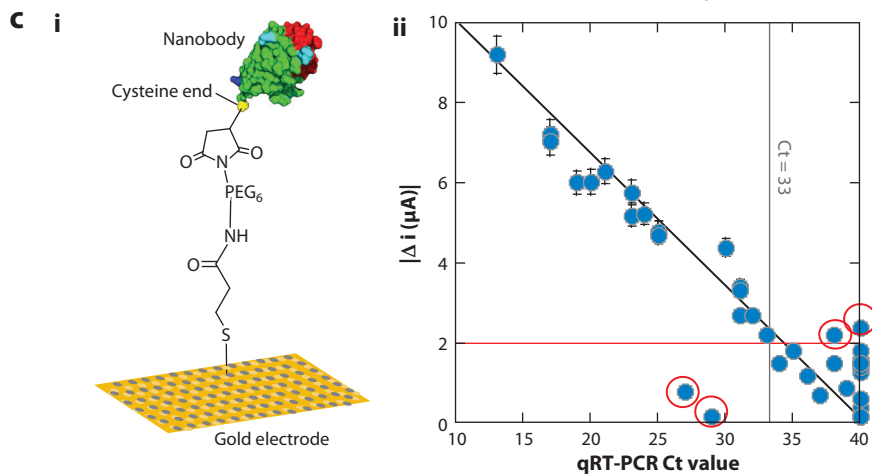
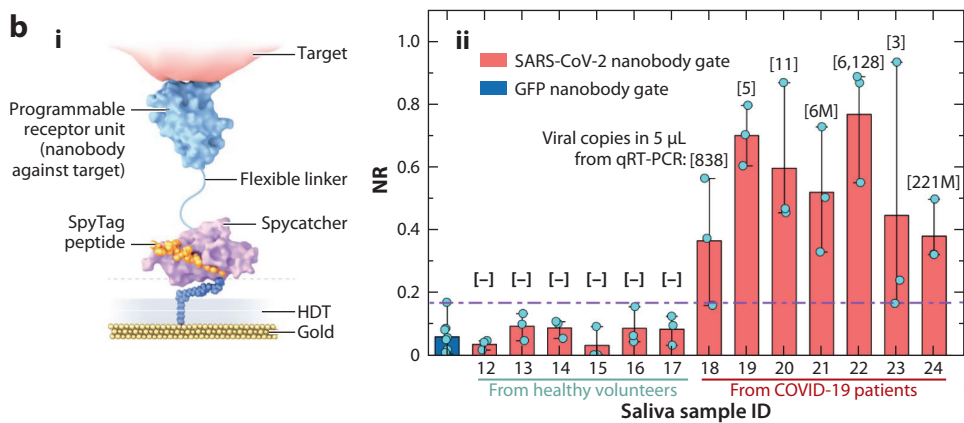
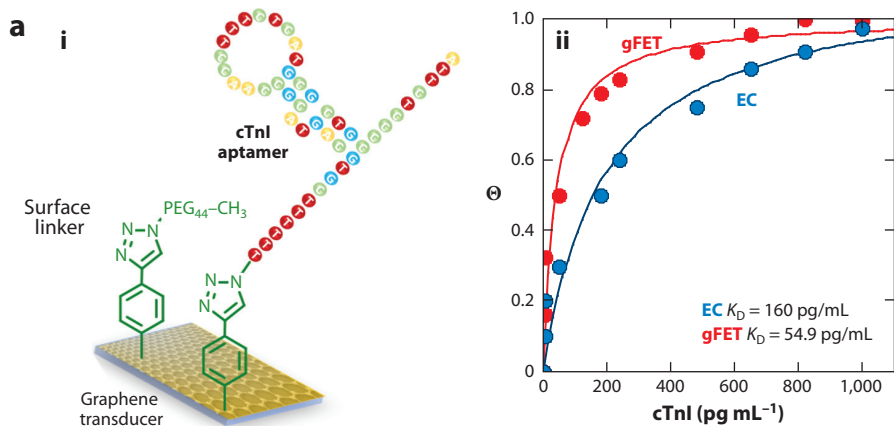
oxidase immobilization without the need for chemical primer. The local pH change triggered by the enzyme-catalyzed oxidation reaction produced a shift in Dirac point to more negative values due to differences in graphene channel conductivity, resulting in an LOD of 4.1  $\mu\text{M}$  for glucose. The same team reported the effect of amino-phosphate interactions on the biosensor performance of enzymatic FETs (53). Using a poly(allylamine)-modified graphene-modified FET (gFET), a sensitive sensor toward different phosphate anions, such as adenosine triphosphate, orthophosphate, and tripolyphosphate, was achieved. A multilayer polyamine-urease layer also allowed the sensing of urea.

## Detection of Oligonucleotides Using Gene-Sensing Interfaces

Electrochemical techniques have been used for genetic measurements for more than 60 years (54). The possible electrochemical activity of the nucleic acid molecules that compose the gene itself was the focus of the first studies in this field. Subsequently, the use of oligonucleic acid molecules linked to electrodes to form complementary bonds (hybrids) with target analytes was behind creating useful DNA sensors (55). These early amperometric methods based on electron transfer changes were later complemented by gate potential changes in FETs. Bashir and colleagues (56) used a deformed monolayer graphene channel to detect nucleic acids, demonstrating the use of FETs for sensing DNA in human serum samples down to 20 aM. The low sensitivity is due to the use of a crumpled graphene sensing channel, and a large part of the charged DNA strand is within the Debye length. More electric charge is detected by the graphene sensing channel, resulting in higher changes in the electric conductance of the graphene channel. Ganguli et al. (57) showed that such devices detected physisorbed single-stranded DNA molecules preferentially over double-stranded DNA products. The discrimination power of crumpled graphene was used to detect *Escherichia coli* DNA down to zeptomolar concentrations. Such a platform will help bring complex laboratory-based diagnostic and genotyping amplification assays to the point of care.

## DNA Aptamer as the Perfect Bioreceptor

In the search for the so-called perfect bioreceptor, chemical antibodies, also known as aptamers corresponding to oligonucleotide sequences with a length of  $\sim 25$ –80 bases that allows them to bind to specific target molecules, were suggested more than 30 years ago as a bioreceptor alternative (58, 59). The secondary structure of aptamers can undergo analyte-dependent conformational changes. This allows a wealth of possible signal transduction schemes to study the kinetic and thermodynamic details of their interactions with the target molecules and quantify them with high sensitivity. While the interest of aptamers for electrochemical and electrolyte-gated FETs has been demonstrated in numerous articles (1, 60–64), uptake in the clinical environment has been slow, mainly due to the absence of standardized protocols for their characterization. A systematic investigation of the use of the same aptamer for electrical and electrochemical-based sensing of cardiac troponin I (cTnI) was recently performed by our research group (63, 65) (**Figure 4a**). The analytical performance of both sensors was found to be comparable, with an LOD of  $5.7 \pm 0.6$   $\text{pg mL}^{-1}$  (electrochemical) and  $3.3 \pm 1.2$   $\text{pg mL}^{-1}$  (electrical). Both sensors have thus a threshold for diagnosing relevant levels of cTnI and the capability to distinguish between low, medium, and severe levels of cTnI in serum samples from patients (63). Surprisingly, different equilibrium dissociation constant ( $K_D$ ) values between the aptamer-linked surface receptor and cTnI were determined in  $0.01 \times \text{PBS}$ . They were 160  $\text{pg mL}^{-1}$  for the electrochemical, which agreed with recent surface plasmon resonance (SPR) results ( $K_D = 176$   $\text{pg mL}^{-1}$ ) (66), and 51.4  $\text{pg mL}^{-1}$  for the gFET transducer (**Figure 4a**). This difference is believed to be related to the use of a redox mediator in the electrochemical sensor for readout and to the ability to diffuse from the solution to the surface via the cTnI/aptamer interface.



(Caption appears on following page)

**Figure 4** (Figure appears on preceding page)

Aptamers and nanobodies for sensing. (a, i) Surface architecture based on a cTnI aptamer and PEG ligand (ii) Langmuir analysis of cTnI binding extracted as a function of the analyte concentration in  $0.01 \times$  PBS (pH 7.4) for the electrochemical concept using ferrocenemethanol as redox mediator and upon application of a fixed gate voltage;  $V_G = 350$  mV for the gFET format. Panel a adapted with permission from Reference 65; copyright 2022 Elsevier. (b, i) Schematic of the nanobody-functionalized organic electrochemical transistor sensor with a nanobody-Spycatcher fusion protein attached to the gate as a bioreceptor. (ii) Average sensor response for saliva samples ( $n = 13$ ) from healthy volunteers (12–17) and patients who had recently tested positive for COVID-19 (18–24). qRT-PCR was performed on each sample, and the viral copy numbers in the  $5\text{-}\mu\text{L}$  sample volume are indicated above the bars. Measurements with one GFP nanobody gate per COVID-19-positive sample served as an additional negative control. The response was calculated as  $\text{NR} = (I_D - I_0)/I_0$  (the current change normalized to the baseline signal, indicated by blue dots). Error bars show the s.e.m. from three gate electrodes measured on one channel. Image in panel b provided by S. Inal. (c, i) Schematic of the nanobody-functionalized electrochemical sensor. (ii) Graph illustrates the correlation of qRT-PCR Ct values with change in electrochemical current from the same patient sample recorded on a gold electrode modified with nanobody VHH 72-13C. The current cutoff value for positive samples was set to  $2\ \mu\text{A}$  (red line). Each patient sample was performed on a new electrode. The results are expressed as the mean  $\pm$  SEM of 3 independent measurements for each sample. Red circles indicate wrongly identified sample results. Panel c adapted with permission from Reference 10; copyright 2022 Nature. Abbreviations: Ct, cycle threshold; cTnI, cardiac troponin I; EC, electrochemistry; gFET, graphene-based field-effect transistor; GFP, green fluorescent protein; HDT, 1,6-hexanedithiol; M, million; NR, normalized response; PBS, phosphate-buffered saline; qPCR, quantitative polymerase chain reaction; qRT-PCR, quantitative real-time polymerase chain reaction; s.e.m., standard error of the mean;  $V_G$ , gate voltage.

## From DNA Aptamer to Peptide Nucleic Acid Aptamers

DNA aptamer–ligand interactions are dictated not only by the secondary structure, but also by noncovalent interactions that the nucleic acid is capable of installing with the target, such as electrostatic interactions, cooperative hydrogen bonding, stacking interactions, or hydrophobic interactions. The overwhelming role of electrostatic interactions can result in a strong dependence on the ionic strength and pH of the sensing medium. DNA aptamers are indeed sensitive to changes in pH, with increased affinity for the cTnI aptamer at more basic pH ( $K_D = 552\ \text{pg mL}^{-1}$ ) and acidic pH ( $K_D = 360\ \text{pg mL}^{-1}$ ) (66). The use of neutrally charged peptide nucleic acid (PNA) aptamers, the synthetic DNA analogs in which the sugar–phosphate backbone of the natural nucleic acids are replaced by a neutral peptide-like backbone composed of repeated *N*-(2-aminoethyl) glycine unit substitutes (67), was first considered by the Knoll and Corradini team (66) for cTnI detection on an electronic device. The superiority of PNA aptamer gFET sensing over DNA aptamer sensing in terms of assay sensitivity was limited by LODs of  $3.34 \pm 0.69\ \text{pg mL}^{-1}$  (DNA aptamer) and  $5.95 \pm 1.02\ \text{pg mL}^{-1}$  (PNA aptamer). Besides the nontrivial demonstration that a PNA can behave as an aptamer, the PNA bioreceptor proved to be more consistent when working in more complex biological matrices, as the  $K_D$  values remained comparable over large ranges in pH and ionic strength. Because PNAs have many applications in diagnostics and therapeutics, these results can form the basis for envisaging a PNA scaffold to study other PNA/protein interactions. While the use of PNA for FET-based sensing is still in its infancy, PNA-based electrochemical sensors are more widely employed (68). An electrochemical PNA-based paper sensor for sensing the PNA/miRNA-492 adduct was proposed by Moccia et al. (69), with a 6-nM LOD and excellent selectivity toward both one- and two-base mismatches and scrambled sequences.

## Peptides as a Promising Approach as a Bioreceptor

Representing an additional promising approach as a biorecognition element, peptides are increasingly being investigated in connection with electrochemical biosensors. The benefits of short peptides mainly consist of good stability and selective affinity to a target analyte. Therefore,

peptide-based electrochemical biosensors represent an alternative approach for the detection of different protein biomarkers (70–72). The possibility to add antifouling properties (**Figure 3a**) into their structures makes this approach of high interest for sensing in complex solutions.

## FROM APTAMERS TO ANTIBODY MIMETICS

Using a full-length antibody (~150 kDa) might be detrimental to optimal electrochemical detection, as the distance from the binder's attachment point to the antigen-binding site is large and might result in diffusion-limited access of redox mediators to the surface. In addition to aptamers, correctly folded antibody fragments such as single-chain variable fragments (scFvs), nanobodies, or nano-clostridial antibody mimetic proteins (nanoCLAMPs) are being considered as alternatives to traditional antibodies in electrical and electrochemical formats (10, 73–76). These antibody mimetics have several desirable characteristics, including low molecular mass, high physicochemical stability, high solubility, and chemical stability. They can be produced in a cost-effective way and have the potential to address the high cost associated with monoclonal antibodies. An improved shelf-life over antibodies with retained antigen-binding capacity makes them ideal building blocks for sensing devices.

Studies utilizing scFvs instead of full antibodies as receptor molecules on FET sensors have shown a thousand-fold improvement in detection limit (77). The label-free electrochemical detection of an *Entamoeba histolytica* antigen using scFv probes was reported 11 years ago (78). This method found wider interest in electrochemical sensing formats more recently (79), even though their use remains limited in general.

Recently, a new class of antibody mimetics called nanoCLAMPs has been described (74, 76), with attractive properties for the development of sensors: These 15-kDa binders can be screened from a synthetic, naive phage display library of  $1 \times 10^{10}$  variants for high specificity and low nanomolar affinity to identify targets in only six weeks. They are naturally devoid of cysteines, are easily refolded following chemical denaturation with 6 M GuHCl, 0.1 N NaOH, or dimethylformamide (DMF) when conjugated to solid support, and have high thermal stability ( $T_m > 65^\circ\text{C}$ ), highlighting their stability in harsh environments. Importantly, they are easily multimerized, making them well-suited countermeasures to quickly mutating targets.

Inal's group used the concept of a flexible SpyTag/SpyCatcher (80) nanobody architecture on 1,6-hexanedithiol-modified gold gates for the sensing of COVID-19 and Middle Eastern Respiratory Syndrome (MERS) antigens in nasopharyngeal and saliva samples (73) (**Figure 4b**). Using the surface-oriented attachment of nanobody VHH-72-13C on a PEG-modified gold electrode via maleimide-thiol linkage chemistry (**Figure 4c**) could reliably and specifically detect SARS-CoV-2 viral particles with differential pulse voltammetry and ferrocene as redox mediator. Its LOD was  $1.2 \times 10^4$  viral RNA copies  $\text{mL}^{-1}$ , corresponding to a Ct value of 33 using cultured SARS-CoV-2 virus particles, which correlates to approximately  $2 \pm 1$  PFU  $\text{mL}^{-1}$  (10). Validation in a study on 80 patients' (40 positive, 40 negative) unprocessed nasopharyngeal samples showed a 90% positive percentage agreement (PPA) and 90% negative percentage agreement (NPA), as compared to reverse transcription polymerase chain reaction (RT-PCR).

## BIORECEPTOR IMMOBILIZATION STRATEGIES AND THEIR INFLUENCE ON THE PERFORMANCE OF ELECTRICAL AND ELECTROCHEMICAL SENSORS

Several surface chemistry approaches are used to link the bioreceptor onto the transducer (71). The importance of optimizing this step has been emphasized by our group's work using a nanobody-based electrochemical sensor (10) (**Figure 4c**) and in the context of cTnI sensing on

aptamer-modified interfaces (63, 65, 81). Although covalent functionalization is by far the most promising approach for reaching robust and stable interfaces (82, 83), using graphene-based FETs generally causes significant change in the electric band structure of graphene. This is due to the rehybridization of  $sp^2$  carbons to  $sp^3$  carbons, not only on the graphene edges but also on the basal plane of the graphene lattice. This has promoted the use of noncovalent modifications mostly relying on  $\pi$ - $\pi$  stacking interactions of aromatic ligands with graphene. The obvious benefit of this approach is that it retains the structures and electrical properties of the graphene (61, 81, 84). Pyrene-based surface ligands have been most widely employed, as van der Waals forces between graphene and the pyrene backbone ensure their tight binding via  $\pi$  stacking, with the use of 1-pyrenebutanoic acid succinimidyl ester (PBASE) becoming particularly popular. Despite the importance of PBASE, the question of whether it is the most suitable surface ligand for obtaining optimal receptor integration via post-functionalization due to the labile character of the reactive ester function was answered in a recent systematic study (81). Comparison with different pyrene ligands showed the limited utility of PBASE due to the labile character. Pyrene carrying a maleimide ending (PMAL) clearly outperformed PBASE anchors, showing full graphene coverage after 2 h without inactivation of the maleimide function. Most interestingly, the time of immersion between graphene and PMAL also had an important impact on the sensing performance toward cTnI. A 2-h incubation resulted in an LOD of  $22 \pm 1$  pg mL<sup>-1</sup>, and a 17-h incubation typically had an LOD of 100 pg mL<sup>-1</sup>.

## WEARABLE SENSORS

With the development of electrochemical and electronic sensing technology, the field of wearable diagnostics is growing and offers innovative solutions to current medical problems (85). Wearable sensors can provide continuous physiological information via noninvasive or semi-invasive measurements of biomarkers in biofluids such as sweat, breath, or interstitial fluid. The ability to simultaneously monitor a broad spectrum of analytes and identify temporal patterns represents great promise as an early warning system following the change of biomarker profiles and might allow the discovery of new biomarkers. Despite the advanced knowledge of biomarker significance and concentration in blood samples for diagnosis, the challenge remains to identify new and specific markers not only in blood but also in other bodily fluids such as sputum, interstitial fluid, sweat, saliva, tears, or breath.

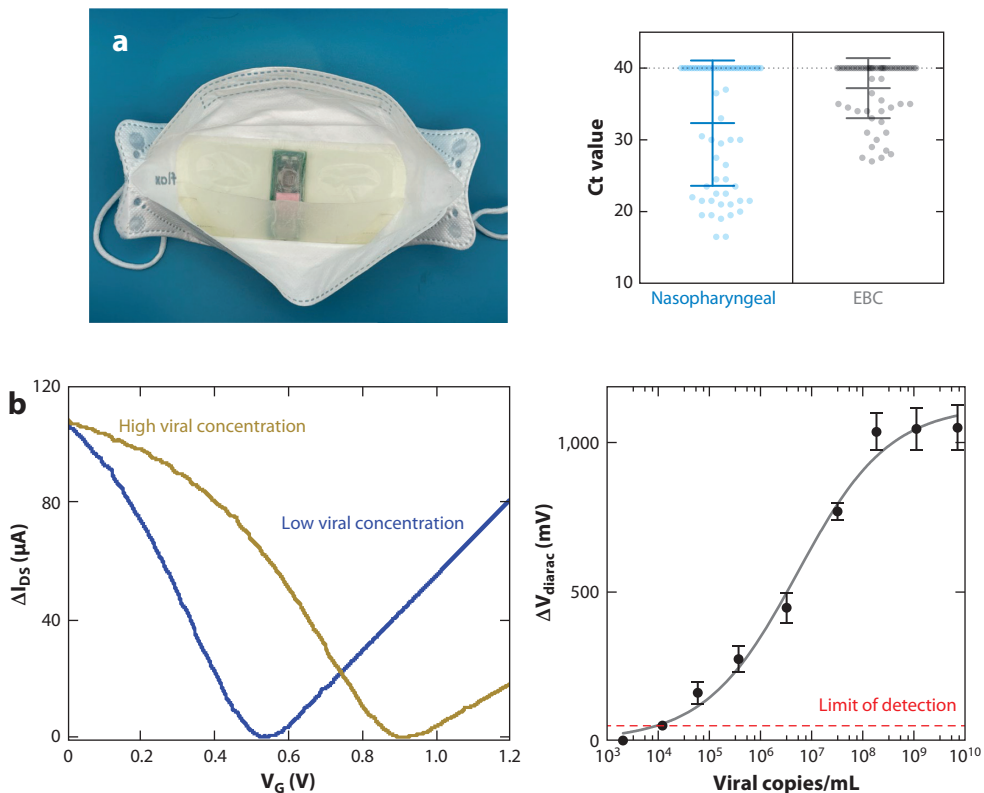
### Breath Analysis

Advancements in modern mass spectrometry instruments allow the encoding of thousands of unique substances in breath, notably different volatile organic compounds, but also peptide and protein components. The field of breathomics has currently advanced to a stage where it can offer solid evidence that breath can be considered as a new diagnostic matrix along with blood, urine, sweat, and saliva.

An example is the identification of the presence of a cardiac biomarker, N-terminal prohormone brain natriuretic peptide (NT-proBNP) in exhaled breath condensate (EBC) (86). Using engineered face masks (1), researchers validated both EBC and blood samples from patients suffering from heart failure secondary to valvular and/or ischemic heart disease during perioperative management for cardiac surgery. ELISA testing also showed a concentration-related correlation between EBC NT-proBNP and serum level.

The same breathing masks were recently used with RT-PCR and an electrochemical read (1) to sense SARS-CoV-2 viral particles (**Figure 5a**). After having patients breathe for 5 min into the face masks, the collected EBC was analyzed using RT-PCR and an aptamer-based electrochemical sensor. The Ct values measured in the EBC samples are lower compared to those measured in





**Figure 5**

Mask-based sensing. (a) Sensing of SARS-CoV-2 virus particles in EBC of 60 patient samples (30 negative/30 positive) collected with chilled facemasks upon 5 min breathing and the correlation with Ct values from RT-PCR analysis. Out of 30 COVID-19-positive samples from nasopharyngeal samples, 24 matched with EBC samples (80% PPA) using RT-PCR. All of the negative nasopharyngeal samples were identified as negative in the EBC, resulting in a 100% NPA. Panel a adapted with permission from Reference 86; copyright 2023 Springer. (b) Transfer curves of a liquid-gated gFET modified with nanoCLAMP P2712 and titrated against increasing concentrations of patient-derived viral particles (H. Dörfler, J. Daniels, S. Wadekar, D. Ladage, G. Greiner, O. Assadian, R. Boukherroub, S. Szunerits, unpublished results), with the lowest viral concentration being  $3.2 \times 10^6$  viral particles  $\text{mL}^{-1}$  (blue). Abbreviations: Ct, cycle threshold; EBC, exhaled breath condensate; gFET, graphene-based field-effect transistor;  $I_{DS}$ , drain source current; NPA, negative percent agreement; PPA, positive percent agreement; RT-PCR, quantitative real-time polymerase chain reaction;  $V_G$ , gate voltage.

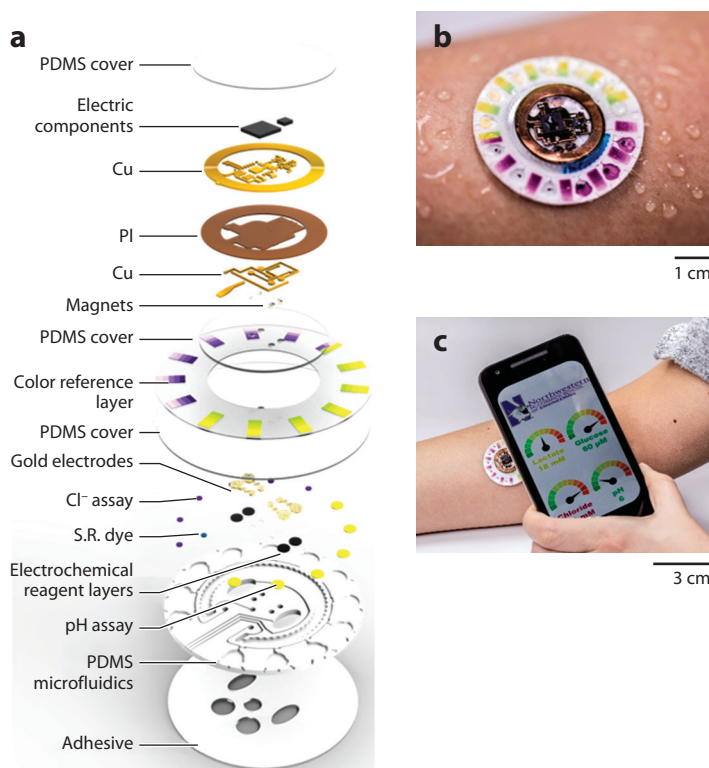
nasopharyngeal samples from the same patient but could be identified in 5 out of 7 cases with the electrochemical aptamer sensor. The sensitivity could be enhanced using a gFET modified with nanoCLAMP P2712 (74). Viral titration experiments showed that the gate voltage shifts with increased viral copies and has an LOD of approximately  $1.2 \times 10^4$  viral copies  $\text{mL}^{-1}$  (correlates to  $\sim$ Ct 33) (Figure 5b). This approach is under development for integration into breathing masks because the gFET sensitivities for SARS-CoV-2 are adapted for this purpose, as outlined recently (87).

### Sweat Analysis

Since the first reports of a textile-based electrochemical sensor for healthcare, sport, and military applications (88, 89), research on wearable electrochemical sweat sensing systems has widely been



conducted and greatly advanced into precise measurement systems. To meet the needs for light weight, small size and portability, and comfortable attachment to the skin, flexible and stretchable materials are used and integrated with electronic modules and wireless communication. These skin-interface systems require a sweat collection (90) and analytical approach. Sweat-stimulating electrodes based on direct iontophoresis as well as transdermal delivery of muscarinic agonists to induce local sweating are the concepts used for sweat collection with ion-selective electrodes, allowing the sensing of potassium or sodium ions in sweat and enzyme-modified electrodes for glucose and lactate sensing (91, 92). An epidermal patch for the simultaneous monitoring of hemodynamic and metabolic biomarkers was recently designed without cross talk between individual sensors (93). Such sensors will not only enrich the understanding of the body's response to daily activities, but can also facilitate the early prediction of abnormal physiological changes. Five years ago, Rogers's team (94) developed a battery-free skin-interface bandage for simultaneous electrochemical, colorimetric, and volumetric analysis of sweat (**Figure 6**). The resulting sensors combine advantages of electronic and microfluidic functionality in a platform that is significantly lighter, cheaper, and smaller than alternatives and demonstrated the possibility to simultaneously monitor sweat rate/loss, pH, lactate, glucose, and chloride. With a two-day human trial, the basis for noninvasive, semi-quantitative tracking of physiological status was provided.



**Figure 6**

Wearable sweat sensors. (a) Schematics of a battery-free sweat sensor, (b) the device during sweating, and (c) the smartphone interface that illustrates wireless communication and data acquisition. Abbreviations: PDMS, polydimethylsiloxane; S.R., sweat rate. Figure adapted with permission from Reference 94 (CC BY-NC 4.0).

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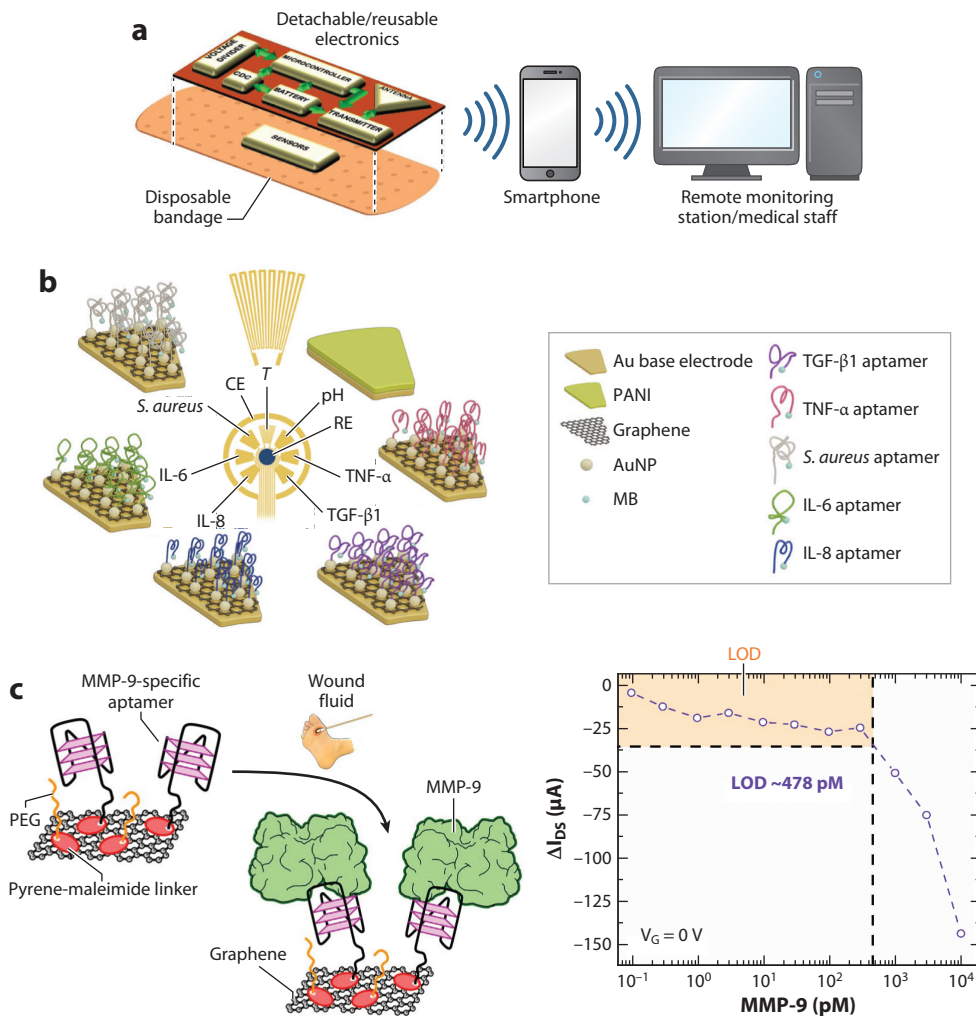
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## Wound Sensors

Although the main focus on wearable skin sensors has been on monitoring physiological molecules, some concepts of this technology are also adapted to other skin-related problems, notably wounds. Chronic wounds, resulting from the failure to undergo the natural healing process, significantly decrease the quality of life and often afflict patients with severe anxiety over the possibility of limb amputations. An increase in both aging populations and patients with diabetes or chronic wounds, such as venous ulcers or diabetic foot ulcers, generates a growing financial burden on the healthcare system. The environmental and physiological factors underlining chronic wounds are reflected in the composition of the wound exudate fluid, which can be used for screening wound infection biomarkers. Wound dressings with integrated pH (95–97) and temperature (97) sensors represent the first approaches to obtain real-time information related to the state of the wound healing process. In work by Farooqui & Shamim (95) (**Figure 7a**), pH levels on the wound site were detected by changes in the resistance of carbon-based electrodes placed on the bandage (95). With a maximum variation in the resistance change due to pH being  $\pm 2.6\%$  and pH values in chronic wounds between 5.4 and 8.6, the variations were too small to allow accurate evaluation of pH changes. Potentiometric pH sensors integrated into wound bandages, based on carbon/polyaniline (PANI) working electrodes, were proposed by Khademhosseini's group (97). The sensors exhibited a relatively wide linear response, with an average sensitivity of  $-50 \text{ mV pH}^{-1}$ . A flexible multiplexed approach (**Figure 7b**) for in situ wound monitoring that integrates temperature and pH sensors with biosensors measuring inflammatory biomarkers, tumor necrosis factor- $\alpha$ , and the presence of *Staphylococcus aureus* was recently reported, using a microfluidic wound exudate technique (60). Indeed, sensors of wound biomarkers produced during the inflammatory and proliferation stage of wounds would represent the intelligent wound-healing bandages of the future. Our team recently validated that zinc-dependent endoproteases, known as matrix metalloproteinases (MMPs), are upregulated in nonhealing wounds. MMP-9 showed values of 1.5–912 pM (61), whereas MMP-9 levels in wounds of a normal person were  $< 1.5 \text{ pM}$ . An MMP-9 aptamer-modified gFET transducer showed sensitivity toward MMP-9 down to 478 pM in simulated wound fluids (98) (**Figure 7c**), representing one step further toward progress in real-time monitoring of the wound-healing stage. The integration of various external stimuli into wound bandages (e.g., heat, electrical fields) (99) is expected to improve the sensitivity of this approach via the change in intermolecular forces between the bioreceptor and MMP-9 analyte. These considerations, mostly overlooked, need to be incorporated into the final device for emerging wearable bioaffinity diagnostics to succeed.

## CONCLUSIONS

Technological and nanoscience advances play fundamental roles in the development of the next generation of electrochemical and electrical biosensors that are cost-effective, accurate, sensitive, and easy to use. Integration of nanomaterials into these diagnostic sensing platforms allows researchers to tackle current sensor challenges related to electrode antifouling. Engineering of antibodies has resulted in more stable bioreceptors with increased shelf-lives of their sensing interfaces. The possibility to reduce bioreceptor size without losing affinity binding to the analyte of interest further opens new possibilities for innovative sensing on electrical transducers. Research in this direction is ongoing but needs to be further validated for its efficiency for electrochemical and electrical biosensors in the future. Other technological efforts have been made to reach increased endpoint detection sensitivities and lower detection limits while preserving selectivity and fast response times using wearable sensors in combination with artificial intelligence. Despite the great promise of wearable sensing strategies, small analytes have been the main target of



**Figure 7**

Intelligent wound bandages. (a) Image of a smart bandage sensor printed on a disposable bandage strip. The wireless electronics, including an inkjet-printed circuit board and antenna, are made on a Kapton tape that can be detached and reused on another bandage. The bandage can communicate wirelessly to a patient's smartphone. Panel a adapted with permission from Reference 95; copyright 2023 Nature. (b) VeCare prototype for chronic wound monitoring with readout of the four immunosensor signals with a wireless portable analyzer fabricated on a FPCB and schematic of the immunosensor for detection of TNF- $\alpha$ , IL-6, IL-8, TGF- $\beta$ 1, *Staphylococcus aureus*, pH, and temperature (T). Panel b adapted with permission from Reference 60; copyright 2021 AAAS. (c) MMP-9 sensing in swab samples from patients with diabetes and open wounds using a liquid-gated gFET aptasensor. Change in  $I_{DS}$  upon increasing concentrations of MMP-9 in simulated wound fluid (5.8 g NaCl, 3.3 g NaHCO<sub>3</sub>, 0.2 g KCl). Orange-shaded area shows the LOD area. Panel c adapted with permission from Reference 98; copyright 2023 Elsevier. Abbreviations: AuNP, gold nanoparticle; CE, counter electrode; FPCB, flexible printed circuit board; gFET, graphene-modified field-effect transistor;  $I_{DS}$ , drain-source current; IL, interleukin; LOD, limit of detection; MB, methylene blue; MMP-9, matrix metalloproteinase-9; PANI, polyaniline; RE, reference electrode; TGF- $\beta$ 1, transforming growth factor beta 1; TNF- $\alpha$ , tumor necrosis factor alpha.

analysis, with the sensing of protein biomarkers and bacteria only starting to emerge in wearable sensor technologies. The field of online sensing via portable sensors still faces serious challenges, such as sensor regeneration and extending their use over one-time sensing. The ability to change the intermolecular forces between the bioreceptor and targeting analyte through various external stimuli (e.g., heat, electrical fields) integrated into wearable electronics needs to be part of the final device for emerging wearable bioaffinity diagnostics to evolve.

Advancements in Internet of Things (IoT) approaches present tremendous opportunities for developing POC sensor technologies via integrating and miniaturizing analytical tools for remote and wireless personalized health monitoring. Defined as the technology connecting multiple devices by means of the Internet, IoT facilitates the transfer of data and information from one device to another. As personalized diagnostics becomes more widely considered, the demand is increasing for analytical devices that continually monitor health conditions of a patient when they are not at the hospital. As modern information and communication technologies are embedded into the healthcare system and personalized IoT-supported wearable devices become available, more efficient healthcare will be provided to elderly patients and those with chronic diseases. However, major challenges posed by IoT devices include their lack of data protocols and security requirements.

Multiplexed sensors are particularly valuable in the continuous acquisition of data related to patients' health, with data comprising multivariable, nonlinear physiological patterns that cannot be readily identified by conventional processing methods. Machine learning has already proven to be an effective tool in the medical field for interpreting large volumes of data. For example, Macchia et al. (100) recently reported on a handheld intelligent bioelectronic system for fast and reliable immunometric POC sensing with integrated artificial intelligence based on binary classification at the limit of identification of a single marker/virus in 0.1 mL. The diagnostic sensitivity, specificity, and accuracy reached 99.2%, as validated through 240 assays, including a pilot clinical trial.

In conclusion, nanotechnology can play an important role in the future of electrochemical and electronic sensing when the focal point on clinical utility and acceptance is taken into consideration. Excess focus on LOD should be avoided. However, future attention should be focused on other analytical performance parameters such as batch-to-batch viability, upscaling possibilities, and reproducibility if reaching better sensitivity has diagnostic merit. Benchmarking of novel electrical and electrochemical diagnostics against clinically accepted gold standard assays, mostly ELISA-based and PCR-based assays, should become standard practice even in high-level academic innovations to make many of the published concepts useful for commercial purposes.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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