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Graphene field-effect transistors as bioanalytical sensors: design, operation and performance†

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Graphene field-effect transistors (GFETs) are emerging as bioanalytical sensors, in which their responsive electrical conductance is used to perform quantitative analyses of biologically-relevant molecules such as DNA, proteins, ions and small molecules. This review provides a detailed evaluation of reported approaches in the design, operation and performance assessment of GFET biosensors. We first dissect key design elements of these devices, along with most common approaches for their fabrication. We compare possible modes of operation of GFETs as sensors, including transfer curves, output curves and time series as well as their integration in real-time or *a posteriori* protocols. Finally, we review performance metrics reported for the detection and quantification of bioanalytes, and discuss limitations and best practices to optimize the use of GFETs as bioanalytical sensors.

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1. Introduction

Bioanalytical sensors, engineered at the interface between physics, chemistry, biology and nanotechnology, are a class of instruments designed for quantitative analyses of biologically-relevant molecules (*e.g.* nucleic acids, proteins, metabolites, drugs, *etc.*). Such biosensors have numerous applications in a variety of areas including biomedicine,^{1–3} environmental monitoring^{4,5} and public health.^{6,7} Analyte detection and transduction into signal can be mediated by different mechanisms, including optical, electrochemical, electrical or mechanical. In the past decades, advances in the field of nanotechnology have catalyzed remarkable innovation in these different subclasses of bioanalytics sensors, especially through the discovery and production of new nanomaterials. For



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example, gold nanoparticles (AuNPs) and inorganic quantum dots (QDs) have been used in the design of ultrasensitive electrochemical⁸ and optical⁹ biosensors. Materials engineering at the nanoscale has enabled artificial nanopores in solid-state membranes (e.g. Si₃N₄ membrane, SiO₂, SiC and Al₂O₃ films) capable of registering the translocation of individual DNA molecules.¹⁰ Nanocarbon materials such as carbon nanotubes (CNTs) and graphene have also stimulated improvements in optical,^{11,12} electrochemical^{13,14} as well as in MEMS/NEMS (micro/nanoelectromechanical systems) bioanalytical sensors.¹⁵

A specific class of nanomaterial-enabled bioanalytical sensors are field-effect transistors (FETs). In FET biosensors, or bioFETs, the interaction with biological analytes is transduced as a change in the electrical conductance of the sensor. The use of FETs for bioanalytical sensing purposes first appeared around 1980, usually adapted from ion-sensitive field-effect transistors (ISFETs) made for pH sensing.¹⁶ For example, Caras and Janata¹⁷ introduced a penicillin-sensitive bioFET assembled by immobilizing specific enzymes on the surface of an ISFET. Early FET sensors were made using traditional semiconductors (e.g. Si) and oxides (e.g. Ta₂O₅ or Al₂O₃), and were often limited in sensitivity due to their low surface-to-bulk ratio. The discovery of low-dimensional semiconductors with extremely high surface-to-bulk ratios prompted the design of various highly-sensitive FET sensors for the detection of ions and molecules.^{18,19} Among these, silicon nanowire FETs (Si-NWFETs) and carbon nanotube FETs (CNTFETs) have both been extensively demonstrated as bioanalytical sensors^{20–22} and even ultimately miniaturized into single-molecule FETs with biomolecules.^{23–25} Despite good sensing performance, the development of 1D-FETs remains hindered today by practical challenges in the synthesis, manipulation and scalable integration of 1D nanomaterials. On the other hand, 2D semiconductor nanomaterials also benefit from extreme surface-to-bulk ratio, but are much more compatible with established microfabrication processes. While a plethora of van der Waals materials have

been discovered in the past few years,^{26,27} graphene is by far the most available and well-studied specimen among them. Since the isolation of individual graphene sheets from graphite by Novoselov and Geim in 2004,²⁸ graphene has received much attention for its exciting mechanical, thermal and optoelectronic properties.²⁹ In particular, graphene was found to exhibit extremely high charge carrier mobility, as well as remarkable sensitivity to electrostatic changes in its near environment,^{30,31} making it a promising material for sensing applications.

In this review, we focus specifically on graphene field-effect transistors (GFETs) as bioanalytical sensor. GFETs have been demonstrated as sensors in physics and chemistry, for instance as photodetectors,³² gas sensors (e.g. NO₂, NH₃, H₂O)^{33,34} or pH sensors.^{35,36} More recently, GFETs have been introduced as biosensors: for instance, Mohanty *et al.*³⁷ reported in 2008 a GFET biosensor able to detect the hybridization between a tethered single strand of DNA and its complementary sequence. Since then, intensive research has been focused on developing GFETs for biomolecular detection. In the bioanalytical field, GFETs have generated interest as ion sensitive field-effect transistors (ISFETs), especially for the detection of toxicology-relevant ions such as heavy metal ions (e.g. Hg²⁺, Pb²⁺).^{38,39} They have also been shown to detect multiple biologically-relevant molecules such as glucose,⁴⁰ various biomarkers for diseases including cancer,^{41,42} DNA sequences with single-nucleotide mismatch specificity,^{43,44} pathogens such as bacteria^{45,46} and viruses,^{47,48} or drugs like opioids⁴⁹ or antibiotics.⁵⁰ GFETs are often described as having key advantages for biosensing applications, including easy operation, fast response,⁵¹ real-time monitoring,^{52–54} high specificity and sensitivity with detection limits down to the femtomolar^{55,56} and sub-femtomolar range,^{57–59} microfluidic integration^{60–62} and multiplexing capability.^{63–65}

In recent years, there has been several reviews discussing the latest research on graphene and its applications as biosensors.^{66–71} However, there is still a lack of a comprehensive review about GFETs focusing on key parameters for asses-



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sing their design, operation and performance, which is essential to progress towards the standardization of this technology and its uptake in industrial, commercial and/or clinical applications. Here we present a critical review of these three aspects of bioanalytical GFET sensors. We cover specifically experiments focusing on the detection of proteins, nucleic acids, bacteria, viruses, small molecules such as glucose, antibiotics or drugs, and heavy ions such as lead, mercury or potassium. We did not investigate pH sensors as they represent a whole field of study by themselves.⁷² In the first part of this review, we briefly explain the fundamentals of GFET operation and review reported approaches for the design and fabrication of such devices. In the second part, we discuss and compare the possible modes of operation of GFETs for the detection and quantitation of bioanalytes. Finally, we review the state of performance metrics reported for this technology and discuss limitations and best practices to optimize the design and performance of GFETs as bioanalytical sensors.

2. Design and fabrication

The design of GFET sensors includes four key components: (1) a graphene layer responsible for the transport of electrical current and the transduction of biosensing events, (2) a set of at least three electrodes as required to operate a transistor, (3) a delivery system allowing tested samples to reach the graphene layer, and (4) a layer of biorecognition elements on the graphene surface allowing for the specific capture of targeted analytes. Fig. 1a illustrates a typical layout for these elements. In the following, we review the role and design principles for each of them.

2.1. Graphene material

Graphene is an atomically-thin material made of a two-dimensional hexagonal lattice of carbon atoms. This structure, with each carbon atom sharing three of its four electrons in

covalent bonds with its nearest neighbors (sp^2 bonds), is at the root of the robust mechanical properties of graphene.⁷³ At the same time, the remaining fourth electrons are delocalized over the two-dimensional lattice in a Π orbital responsible for most of the material's optoelectronic properties.⁷⁴ In the context of GFET sensors, we focus on the electrical and electrostatic properties of the material. Graphene is known for its extremely high mobility surpassing that of excellent metals.^{28,75} Being a semi-metal, its electrical conductance is moderately modulated by local electrostatic fields, allowing to operate the material in a field-effect transistor configuration. Because of this moderate ON-OFF modulation, graphene FETs are typically not considered competitive in pure electronics, compared to state-of-the-art 3D semiconductors such as silicon, or even to its 1D counterpart carbon nanotubes. However, their sensitive electrical conductance combined with their extremely high surface-to-bulk ratio provides them with significant advantages for chemical and biochemical sensing.

Graphene can be produced by several different methods before integration in a FET device. First, graphene can be exfoliated from graphite, a material formed of multiple stacked atomic layers of graphene: the process consists in carefully extracting one monoatomic layer from the bulk graphite. Exfoliation can be achieved by various techniques, including chemical exfoliation,⁷⁶ ball milling method,⁷⁷ or more commonly micromechanical exfoliation, often referred as the "scotch-tape method".⁷⁷ The scotch tape method was the first reported to isolate graphene,²⁸ and typically provides the best electrical properties, including the highest mobilities and least density of defects.⁷⁸ However, it is difficult to obtain large-area flakes with exfoliation, which makes this approach less suitable for large-scale fabrication of devices.⁶⁴ Graphene can also be grown by chemical vapor deposition (CVD), most commonly on metallic substrates like Cu or Ni.⁷⁹ In this approach, a hydrocarbon precursor is introduced at high temperature, leading to graphene nucleation on the metal surface. Epitaxial growth on insulating SiC is also possible, in which case gra-



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For protein detection, the most common strategy is the use of antibodies as probes, due to their high specificity and affinity for their antigen. For instance, GFETs functionalized with antibodies have been used to detect proteins identified as cancer biomarkers: Kim *et al.*⁴¹ immobilized monoclonal antibodies against the prostate specific antigen (PSA) on a GFET biosensor, demonstrating highly sensitive detection of this biomarker of prostate cancer. In a similar way, monoclonal antibodies on GFETs were used to detect alpha-fetoprotein (AFP), a biomarker of hepatocellular carcinoma (HCC), in patient plasma.¹¹⁵ Other studies have used GFETs with antibody probes for biomarkers to other conditions, such as human Chorionic Gonadotrophin (hCG), a common pregnancy indicator.⁸⁹ Antibodies on GFETs have also been shown to detect surface proteins of bacteria^{46,84,90} or viruses.^{47,48,136,137} For example, Chang *et al.*⁸⁴ and Thakur *et al.*⁴⁶ used anti-*E. coli* antibodies in order to detect the bacteria, and more recently Ono *et al.*⁹⁰ used immunoglobulin G (IgG) to immobilize the gastric pathogen *H. pylori* on GFETs. Similarly, Liu *et al.*⁴⁷ used specific antibodies to achieve rotavirus detection. Recently, GFETs with antibodies were also used to detect the SARS-CoV-2 virus responsible for COVID-19.¹³⁶ Antibody probes were also used for the detection of larger complexes such as exosomes⁴² as well as small molecules such as the pesticide chlorpyrifos.⁵⁶

Aptamers are another type of probe molecules used in GFETs; these are folded single-stranded DNA or RNA oligonucleotides that can bind a target protein or small molecule with high affinity and specificity. Saltzgaber *et al.*⁶⁴ functionalized graphene with aptamers designed to bind specifically to human thrombin proteins. Farid *et al.*¹⁰² reported a GFET functionalized with aptamers for detection of the cytokine interferon-gamma (IFN-gamma) associated with tuberculosis susceptibility. Recently, Wang *et al.*⁶¹ studied the binding kinetics of human immunoglobulin E (IgE) to its specific aptamer, allowing the determination of thermodynamic properties of their interaction. In addition, the use of RNA aptamers has been reported for the detection of small molecules, such as the antibiotic tobramycin.⁵⁰

2.4.2. Strategies for probe immobilization. By far the most popular approach to immobilize probe molecules is through graphene functionalization with the linker molecule 1-pyrenebutanoic acid succinimidyl ester (PBASE).^{42–44,48,53,57,61,64,65,81,83,88,90,115,125} On one end, this molecule contains an aromatic pyrene group that binds to the graphene surface through non-covalent π - π interactions. The other end is made of a succinimidyl ester group, which is prone to form a covalent bond with amine groups *via* nucleophilic substitution.¹³⁸ Probes made of DNA are often immobilized with PBASE, usually *via* an amine-terminated modifier attached at the 3' or 5' extremity of the strand. This approach has been reported for simple ssDNA probes,^{53,65,83,139} and also for more complex ssDNA probes,^{43,125} hairpin-ssDNA probes⁵⁷ and dsDNA probes based on strand displacement,⁴⁴ as described in the previous section. Aptamers can also be immobilized with the same approach.^{61,64} It should be noted that Kim *et al.*¹⁰⁸ reported the immobilization of ssDNA

without terminal modifier *via* covalent coupling of the PBASE directly with the amine of nucleobases (adenine, cytosine, and guanine), and of dsDNA *via* non-covalent interactions between the phosphate groups in the DNA backbone and the succinimidyl ester moiety of PBASE. Instead of directly using PBASE, graphene can be functionalized with 1-pyrenebutyric acid, which is then activated using EDC/NHS chemistry into an NHS-ester.¹⁴⁰ In a different approach leading to the same construct, the pyrene moiety is sometimes directly functionalized to the ssDNA as a modifier to the 3' or 5' termination, and the pyrene-DNA complex is then linked to the graphene; this approach was used in Farid *et al.*¹⁰² to immobilize aptamer probes, and in Fu *et al.*¹²⁹ to immobilize ssDNA probe.

The PBASE approach is also frequently used to immobilize proteins, by covalently reacting the succinimidyl ester group with the amine-terminated residue of an amino acid (*e.g.* lysine) available at the surface of the protein. For instance, this approach was successfully applied to immobilize various antibodies^{90,115} as well as the dCas9 enzyme used for detection in genomic DNA in Hajian *et al.*⁵⁵ Some groups use biotin-streptavidin as an intermediary to immobilize protein probes:^{63,90} for example in Ono *et al.*,⁹⁰ amine sites on the urease probes are functionalized with biotin linkers which are then coupled to streptavidin molecules immobilized on graphene with PBASE. A common aspect of these approaches with proteins is that there are frequently multiple available amine sites on a protein, and thus targeting these provides little control on the orientation of the probe on the sensor surface. This distribution can actually be an advantage for sensing by positioning part of the target-binding sites closer to the graphene surface below the screening limit (see section 4.1).¹⁴¹

Graphene can also be functionalized with covalent moieties, which can then be conjugated with biomolecules. A common reaction to do so is through the use of aryldiazonium salts, in which highly reactive radicals formed from reduced diazonium can directly bind to the carbon lattice of graphene.¹⁴² The functionality of the aryl group is chosen for further bioconjugation with biomolecule probes: for instance, 4-carboxybenzenediazonium tetrafluoroborate (CBDT) creates stable carboxyphenyl anchor groups on the graphene surface. These -COOH moieties can then be activated using EDC-NHS chemistry into a stabilized NHS-ester ready for coupling to an amine group on the probe, as described with PBASE above. Lerner *et al.*⁴⁹ used this approach based on CBDT covalent functionalization followed by EDC-NHS reaction to immobilize an opioid receptor protein for naltrexone detection. Others have reported using the EDC-NHS reaction directly on carboxylated defects spontaneously present on the graphene material.¹¹⁷ In a reverse configuration, the functionalization of graphene with primary amines (-NH₂) was shown using electron beam-generated plasmas produced in Ar/NH₃; amine-terminated ssDNA were coupled with the amine-functionalized graphene using glutaraldehyde as a bifunctional linker.¹⁴³

Covalent and non-covalent immobilization approaches have different impacts on GFET sensors. Covalent functionalization causes a significant structural change in graphene: it trans-



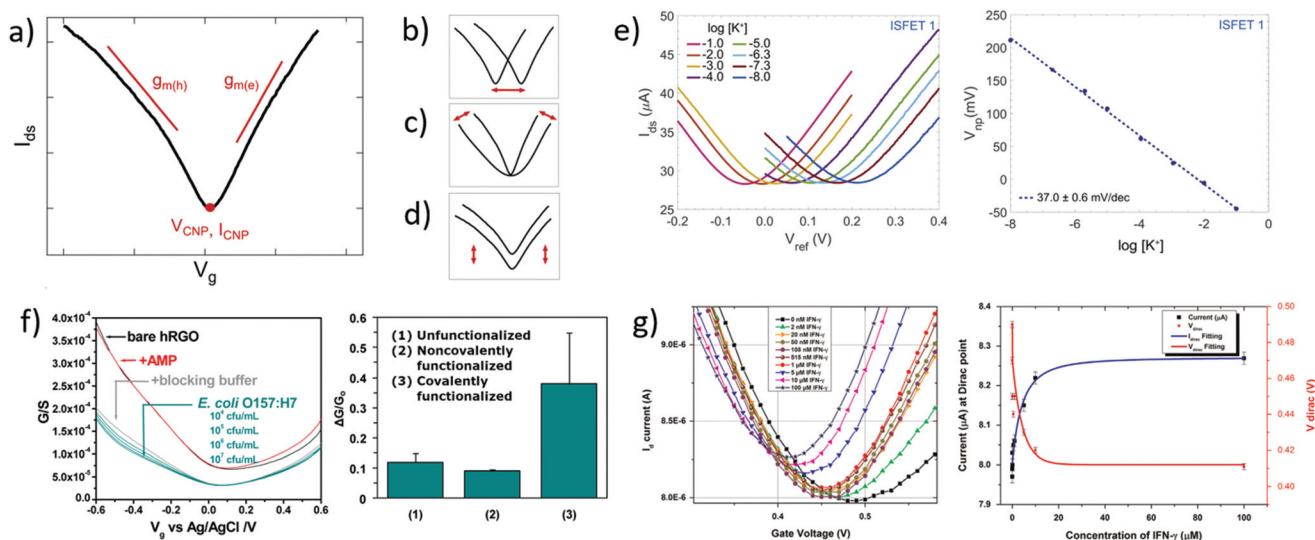


Fig. 2 Transfer curves in GFET bioanalytical sensors. (a) Typical transfer curve I_{ds} (V_g) of a GFET, illustrating key metrics in its use as a sensor: (b) change in the voltage of the charge neutrality point V_{CNP} , (c) change in the transconductance of electrons $g_{m(e)}$ or holes $g_{m(h)}$, and (d) change in the current amplitude, including at the charge neutrality point I_{CNP} . (e) Left: GFET experiment showing a lateral shift of the transfer curve upon exposure to increasing concentrations of its target analyte, here potassium cations. Right: Corresponding shift of V_{CNP} as a function of K^+ concentration. Reprinted with permission from Fakih *et al.*¹¹⁹ © 2019 Elsevier B.V. (f) Left: Experiment with a GFET sensor for *E. coli*, showing a change of transconductance in the p-branch of the transfer curve upon increasing bacteria concentration. Right: Corresponding relative conductance change at fixed bias for different surface functionalization of the sensor. Adapted with permission from Chen *et al.*⁷⁸ © 2014 American Chemical Society. (g) Left: GFET experiment for detecting interferon-gamma protein (IFN- γ), showing a change in all three metrics with exposure to the protein. Right: Response of V_{CNP} and I_{CNP} as function of IFN- γ concentration. Reprinted with permission from Farid *et al.*¹⁰² © 2015 Elsevier B.V.

where V_{CNP} is the gate voltage at the charge neutrality point. The slope g_m is called the transconductance

$$g_m = \frac{W}{L} \mu C_g V_{ds} \quad (2)$$

which depends on the width W and length L of the graphene, μ the mobility of charge carriers and C_g the gate capacitance.¹⁴⁸ Transconductances for holes and electrons are not necessarily the same, in which case the transfer curve is asymmetrical.

Transfer curves can be obtained using any of the three gate electrode configurations described in section 2.2 and illustrated in Fig. 1. The gate capacitance – and thus the transconductance – is highly dependent on this layout. In a back-gate configuration, the gate capacitance is dominated by that of the insulating layer separating graphene from the planar gate electrode, typically an oxide with a thickness t ranging from ~ 10 nm to a few μm . The capacitance of this insulating layer is inversely proportional to its thickness: $C_g \approx C_{ox} = \epsilon_{ox}/t$, with ϵ_{ox} the electric permeability of the dielectric. In the case of immersed or co-planar gate configurations, the shape and position of the gate electrode can vary considerably, but the capacitance is mostly determined by the electrical double layer (EDL) formed at the graphene surface by the reorganization of ions in the electrolyte media. This EDL acts similarly as a very thin dielectric layer – in the range of angstroms to a few nanometers.¹⁴⁹ The resulting gate capacitance is much larger than that of back-gate dielectrics, and can reach levels comparable

to the quantum capacitance C_Q .¹⁵⁰ The gate capacitance is then determined by combining the quantum and EDL capacitances in series: $C_g = [C_Q^{-1} + C_{EDL}^{-1}]^{-1}$.⁶⁶ Gate potentials applied across the EDL can be over two orders of magnitude more efficient than through the back gate: consequently, the sweeping range of gate voltage required to capture the linear p- and n-branches is much smaller for immersed or coplanar gates, typically in the order of ± 1 V,¹⁵⁰ compared to ± 10 V for thin oxides, going up to ± 100 V for thick insulators in the back-gate. In electrolyte media, the range of gate bias sweep must also be restricted to avoid unwanted hydrolysis reactions and other electrochemically-driven reactions at the electrodes.⁶⁶

The choice of gate configuration for a GFET sensor depends on the application. The capture of biomolecular analytes (nucleic acids and proteins) normally occurs during immersion of the probe-functionalized graphene layer in the sample, either an analyte-enriched buffer or a biological sample, such as biomedical (blood, serum, urine, *etc.*), food or environmental. Analyte detection by electrical measurements, though, can occur directly in the same media or after its removal. Immersed or co-planar gate configurations allow electrical measurements directly in electrolytic samples, and are thus usually favored in GFET bioanalytical experiments. The back-gate configuration is generally not used when the GFET interface is immersed with electrolytes, because screening by the EDL can lessen the back-gate voltage. Back-gated GFET sensors are more frequently used for the detection of volatile analytes in gaseous media, for example in applications such as



the detection of pollutants.^{114,151} Nevertheless, back-gated GFETs have been recently reported to detect exosomes directly in buffer using the back-gate by exposing only part of the graphene surface to the sample,⁴² and they also have been used to detect DNA or naltrexone by immersing the device for exposure followed by drying before measurement.^{49,132} Drying the sample is limited to *a posteriori* detection and can result in non-specific adhesion of various species on the sensor surface, so particular attention to specificity should be exerted in this approach. Finally, let's note that the electrical interaction between analyte and graphene could also differ between dry and immersed conditions, as difference in environment are expected to alter screening effects as well as intramolecular charge transport properties.¹⁵²

From transfer curves, several electrical metrics can be used for sensing, as illustrated in Fig. 2b–d and discussed in the following:

3.1.1. Change in CNP voltage. The most commonly used electrical metric in GFET sensing is a change in the CNP voltage value, *i.e.* the gate voltage associated with the minimum of the transfer curve, as illustrated in Fig. 2b. The CNP voltage depends on the doping level of the graphene: for intrinsic graphene at low drain–source bias, it is expected at values close to 0 V, but in reality, it can be either positive if the graphene is p-doped (indicating a larger density of holes) or negative if n-doped (larger density of electrons). The doping level depends on many factors, including the nature of the interface between graphene and other materials (substrate, electrodes, media) and the distribution of charged species and impurities in these materials.¹⁵³ Consequently, the choice of materials in device design, their quality and the different processing steps during fabrication of the GFETs have influence on the initial doping state of the graphene layer. In particular, for GFETs made from CVD-grown graphene, the quality of the fabrication process is sometimes associated with the magnitude of the doping,¹⁵⁴ as it can reflect the quantity of impurities located between graphene and the substrate following the transfer process (see section 2.2).^{83,108} Efforts in reducing contaminants in the transfer process has been shown to bring the V_{CNP} closer to 0 V.¹³² Biosensing experiments based on the change in V_{CNP} can be carried out regardless of the initial doping of the graphene, as long as it is moderate enough to have the CNP visible in the gate voltage sweep at every step of the experiment; otherwise another metric must be used.⁵⁰

In biosensing experiments, the interaction between biological targets and biorecognition elements at the surface of graphene can alter the doping state of graphene, thus creating a shift in the CNP voltage from its initial value. This CNP shift is by far the most common metric for biosensing using GFETs.^{41,42,49} For example, Fakhri *et al.*¹¹⁹ used the shift in CNP voltage as the sensing metric for K^+ ions: they measured transfer curves for a wide range of concentrations of the target ion, as illustrated in Fig. 2e, showing a systematic shift of the curve with analyte concentration. In this experiment, the detection appears to be purely mediated by a doping mechanism, since the whole transfer curve is shifted without altering its

amplitude and slope between measurements. From these transfer curves, a clear linear correlation between the CNP voltage and the log of analyte concentration was demonstrated, also shown in Fig. 2e. The change in V_{CNP} is also used as a detection metric for complex macromolecular analytes such as DNA oligomers. For example, Gao *et al.*⁵⁷ used the shift of the CNP as a sensing metric for 22 nt single-stranded DNA targets binding to hairpin DNA probes. They reported high sensitivity and specificity with this metric, using it to detect single nucleotide mismatches in the target. Finally, the change in CNP voltage is also frequently used to monitor intermediary steps in the assembly of the biorecognition layer, such as graphene chemistry or immobilization of biomolecular probes.^{46,57,104}

The polarity of the CNP voltage shift raises interesting questions. Polarity represents the direction of the change on the voltage axis: p-doping when the CNP shifts to more positive voltage, n-doping when it shifts to more negative voltage. The polarity depends on the interaction between analyte molecules and the functionalized graphene layer. Polarities of the change in CNP voltage are reported in Table 1 for different types of analytes: cations, glucose and DNA. All cation sensors report a negative doping, which is consistent with an electrostatic gating model: the capture of positively-charged targets attracts negative charge carriers in the graphene, generating n-doping and a negative shift of the V_{CNP} .⁶⁶ Oppositely, negatively-charged target molecules would increase the density of holes in graphene and generate a positive shift. This electrostatic gating effect is usually postulated as the mechanism also involved in the detection of molecules; however observations are often inconsistent with this model. For instance, various experiments of GFET sensors for DNA and glucose present opposite polarities in the change of CNP voltage, as compiled in Table 1. For DNA sensors, this discrepancy is associated with at least two opposite effects. Studies observing a p-shift often attribute it to a chemical gating effect, in which the deprotonation of the phosphate backbone of the captured target DNA leaves it negatively charged in buffer, leading to the positive shift.⁵⁸ On the other hand, observations of n-doping are explained by non-electrostatic stacking interactions between nucleotides and graphene,^{43,105} or donor effect,¹⁶² which is supported by DFT calculations.^{163,164} These differences may arise from experiment-specific differences in the graphene–analyte–solution interactions when immersed in

Table 1 Polarity of reported changes in CNP voltage for different analytes in published studies

Target	Doping polarity	Ref.
Cations (K^+ , Hg^{2+} , Pb^{2+})	n–	87, 101, 119–121, 155–158
	p+	40, 91 and 92
Glucose	n–	159
	p+	57, 65, 81, 83 and 92
DNA	n–	43, 44, 59, 60, 63, 104, 105, 107, 109, 125 and 160–162



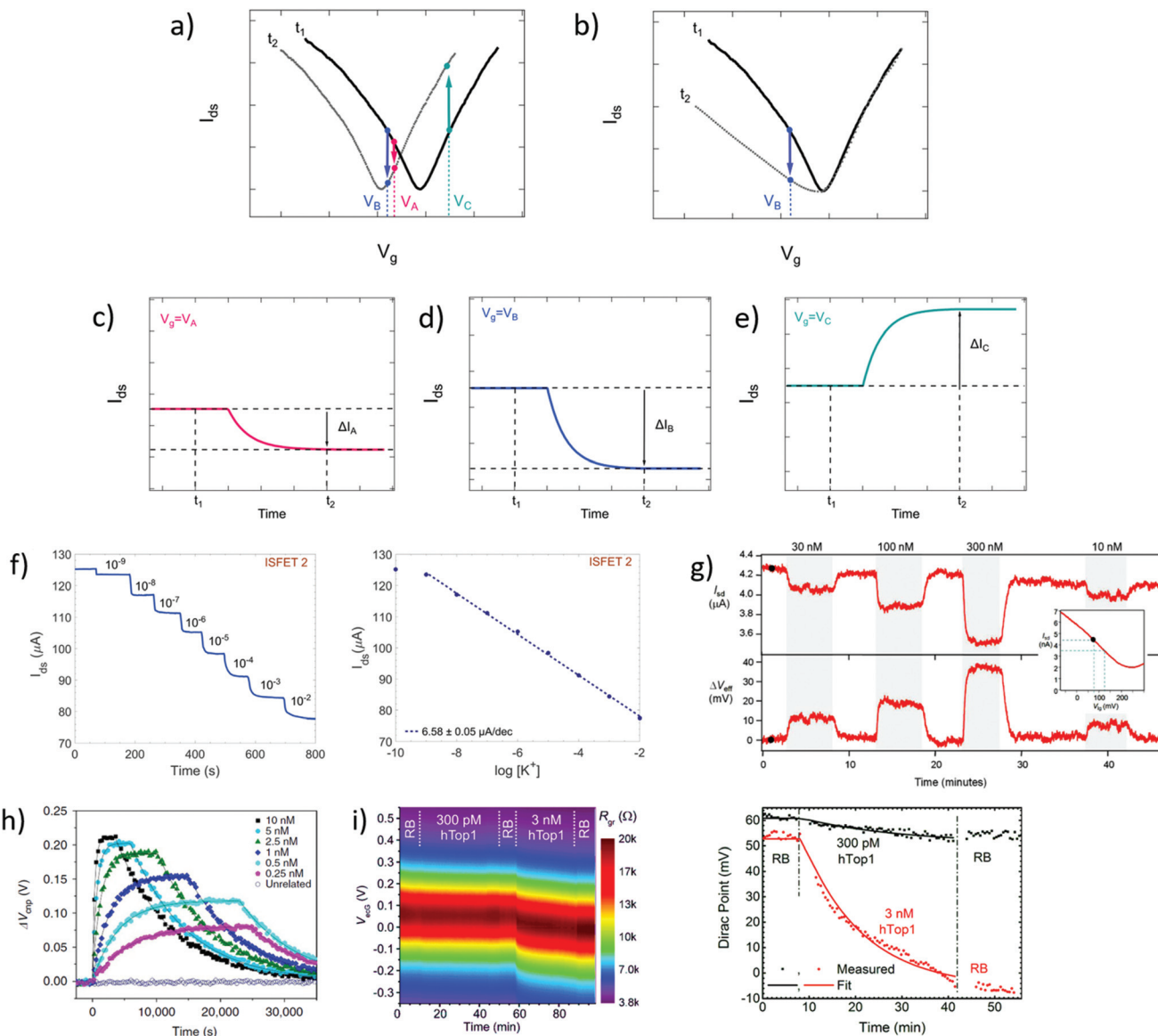


Fig. 4 Time series in GFET biosensors. (a) GFET sensor detecting a left-shift of the CNP voltage, captured in two transfer curves at time points t_1 and t_2 . (b) Same for a system undergoing a change in p-branch transconductance. (c)–(e) Corresponding time series of current $I(t)$ at specific gate voltages V_A , V_B and V_C . (f) Left: Time series of current in a GFET sensor for K^+ ions, recording the exposure to increasing concentrations of analyte. Right: Corresponding change in current as function of K^+ concentration. Reprinted with permission from Fakhri *et al.*¹¹⁹ © 2019 Elsevier B.V. (g) Time series of a GFET sensor for thrombin, recording the introduction of various concentration of analyte separated by washing cycles. Top series shows the current as a function of time, and bottom series the corresponding change in CNP voltage using the conversion described in the inset. Reprinted with permission from Saltzgeber *et al.*⁶⁴ © 2013 IOP Publishing, Ltd. (h) Time series of the change in CNP voltage, also obtained by conversion, showing hybridization and dissociation kinetics between ssDNA probes immobilized on a GFET and different concentrations of the complementary ssDNA. Reprinted with permission from Xu *et al.*⁵³ © 2017 Springer Nature. (i) Left: Two-dimensional time series showing electrical current as a function of both gate voltage and time, here for a GFET sensor targeting the hTop1 enzyme. Right: Time series of the CNP voltage, extracted from the 2D plot, during introduction of hTop1 at two concentrations (right). Reprinted with permission from Zuccaro *et al.*⁵⁴ © 2015 American Chemical Society.

(Fig. 4h). In this approach, the current change ΔI_{ds} is converted to a voltage change with the relation $\Delta V_{CNP} = \Delta I_{ds}/g_m$. It's important to note that this approach is only valid if the transconductance remains constant before and after the addition of targets. As previously mentioned, transfer curves should be provided to confirm that doping is the only mecha-

nism at play. Signal in time series is sometimes normalized as a relative change from a baseline current. Use of normalization can help in assessing signal strength despite sensor-to-sensor variations and effects associated to the medium.⁵⁵ For example, Chen *et al.*⁵⁰ used a simple normalization I_{ds}/I_0 with I_0 the initial current in deionized water and Liu *et al.*⁴⁷ showed



exposure, like in Fig. 4f: such stabilization of the signal facilitated its quantification, which is critical for analyte quantification. A combination of the two purposes, kinetics and quantification, can be done simultaneously, like in Saltzgeber *et al.*⁶⁴ in Fig. 4g, where the successive introductions of different target concentrations, separated by washing steps, are analyzed to gain insight on the effect of concentration on the kinetics of the reaction. Real-time experiments also allow to assess the reaction rate and the time required to stabilize the interaction between the analyte and the target, which can then be used to determine how to time transfer curves for before–after measurements. For real-time biosensing, experiments need to be done in a saline environment with a coplanar or immersed gate configuration, which requires a flow cell or microfluidic circuitry. Measurements can be done in a static or continuous flow setting. Faster reaction times have been reported for DNA sensing in such settings,^{53,134} but continuous flow was reported to lead to noisier signals because of vibrations due to the water pump.⁵⁰ The choice of flow configuration thus depends on the priorities in the experiment.

For most experiment purposes, both types of measurements are best used together. Standard time series are very instructive about the kinetics of the analyte–sensor interactions, but since they only measure the current at fixed biases, they provide little insight on the physical mechanism underlying these interactions. When time series are coupled with transfer curves, either at specific time points or, even better, continuously in two-dimensional time series,⁵⁴ then the mechanisms behind the evolution of the current can be further investigated. In addition, quantitative analyses based on current changes (either for quantitation or kinetics) often rely on the assumption that the change in current is proportional to the change in graphene doping state, but this is only true in the linear regime of p/n branches and if there is no change of the charge carrier mobilities during the reaction; this needs to be confirmed with transfer curves. Finally, when using before–after experiments without any time series, it is difficult to assess whether the interaction with analytes is stabilized or not; consequently, incubation times are often chosen very long in order to make sure the reaction has occurred. The use of time series, at least during calibration assays, could help optimize the incubation time used in detection assays.

4. Performance assessment

The experiments considered in the scope of this review aim at developing GFETs as a bioanalytical technology, *i.e.* for the detection or quantitation of molecules relevant in biology. In this section, we review the criteria used to evaluate the performance of GFETs as biosensors. In this context, performance include two aspects: quality and reliability.¹⁷⁵ Quality criteria are established by the performance of the sensor itself with respect to several detection metrics. In the following, we discuss four of these metrics: spatial range of detection, limit of detection, sensitivity to target concentration and response

time. Reliability criteria can be assessed by the experimental design; here we will discuss appropriate statistical sampling and analysis, as well as controls experiments.

4.1. Spatial range of detection

For electrolyte-gated GFETs, it is important to take into account charge screening by mobile ions in the medium. According to the Debye–Hückel model, charged molecules in solution are screened by mobile counter-ions such that their electric potential is dampen exponentially with distance, with a decay constant λ_D called the Debye length. This constant represents the screening length and is given by

$$\lambda_D = \sqrt{\frac{\epsilon k_B T}{2N_A e^2 I}} \quad (3)$$

where ϵ is the permittivity of the medium, k_B the Boltzmann constant, T the temperature, N_A the Avogadro's number, e the electron charge and I the ionic strength of the solution. The ionic strength is given by $I = \frac{1}{2} \sum_i \rho_i z_i$ where ρ_i and z_i are respectively the density and valence of ion species i . Generally speaking, the Debye length represents the distance at which charges are screened; thus, charges located farther than the Debye length are usually considered out of range for electrostatic detection by a FET sensor.^{176–178} For an aqueous solution at room temperature, this length becomes λ_D (nm) = $0.304/\sqrt{I}$ where I is in mol L⁻¹. For 1× PBS buffer, it is as short as ~0.7 nm. Therefore, one must take λ_D into consideration when designing specific probe molecules, as too-long a distance between target binding events and the FET surface may significantly reduce the signal^{146,178–180} or completely screen it out.^{146,176} Such limitations due to Debye length on the spatial range of detection in FET sensors have been experimentally observed in different types of FETs. For example, Sorgenfrei *et al.*¹⁷⁸ used an ssDNA probe tethered to a CNTFET to study the effects of shortening the Debye length, *via* changing PBS concentration, on the detection of probe hybridization with a target complementary DNA (cDNA). They found the resistance to decrease significantly (resistance change $\Delta R/R$ dropping from 80% to 10%) when increasing buffer salinity from 0.1× to 5× PBS (corresponding to a decrease of λ_D from 2.3 nm to 0.3 nm). They also showed that moving the target cDNA further from the surface, by removing two base pairs from the target cDNA (~0.66 nm distance increase), reduced $\Delta R/R$ ~ from 80% to 20%. This was performed in 1× PBS and, notably, a signal was still detectable, although greatly reduced, even though hybridization occurred at distance of ~1.36 nm, exceeding the estimated λ_D of 0.7 nm.

This proximity requirement between the captured analyte and graphene presents a challenge in designing the interface of GFETs, especially in biomedical applications targeting detection in physiological samples. Saline buffers such as 1× PBS or 1× PB, commonly used to emulate physiological environments (*e.g.* human blood), have a very short Debye length of 0.7 nm. In comparison, common probe molecules





Fig. 5 Sensitivity and limit of detection (LOD) in GFET biosensors. (a) Typical calibration curve for a GFET sensor, showing the change in a given electrical metric as a function of analyte concentration. Sensitivity represents the slope of the linear regime, while the LOD is the concentration at which the change in the metric exceeds a chosen confidence interval. (b–c) Methods for the experimental determination of the LOD based on extrapolation and direct measurement, respectively. (d) LODs reported in the literature for GFETs, classified by analyte type: ions, small molecules, DNA and proteins. Data points are also separated as function of the type of graphene used in GFET fabrication (ME = mechanical exfoliation, CVD = chemical vapor deposition, rGO = reduced graphene oxide). (e) Reported LODs for DNA detection represented as function of the length of the targeted DNA sequence.

positively on signal strength and is limited by noise and other sources of variance in the measurements. Although distinct, the LOD and sensitivity can be correlated. For instance, in Fig. 5a, we can see that improving the sensitivity or the slope in the calibration curve is likely to lower the LOD. The LOD is relevant for detection and also for quantitation of analytes: it determines the lower bound of the dynamic range of the sensor, the upper end being limited by the saturation of the signal. In practice, the required LOD depends on the purpose of the sensor: for some applications, a predefined LOD needs to be achieved as prescribed by norms or regulations, for example safe Pb^{2+} levels in children's blood¹²¹ or glucose levels associated with diabetes.¹⁵⁹ In the development of GFET biosensors, the LOD is the most frequently reported indicator of performance, and is often benchmarked against other detection techniques, such as PCR-based techniques for DNA detection,⁸¹ or ELISA tests for immunoassays.¹⁷⁴ We surveyed the literature on GFETs used as bioanalytical sensors and retrieved 61 studies reporting a value for the LOD (see the ESI† for a list of the studies). An interesting observation from that compilation is that the reported LODs cover a very wide range of concentrations, from 0.1 mM (ref. 91) to 8 zM,¹²⁴ which represents a remarkable spread of 16 orders of magnitude. In the following, we discuss the different factors that can influence the LOD in GFETs.

First, we note that methodologies to determine the LOD vary between studies. They can usually be classified in one of two categories: extrapolation^{53,134,189,190} or direct measurement,^{41,49,65,89,120,121,174} illustrated in Fig. 5b and c respectively. Both approaches first require precise characterization of the sensor baseline, *i.e.* the value and standard deviation of the chosen electrical metric (ex. CNP voltage, current) in absence of the targeted analyte. The LOD differs from the baseline of the sensor by an interval of confidence, defined as a chosen multiple of the standard deviation. The International Union of Pure and Applied Chemistry defines the LOD to be at three times the standard deviation,¹⁹¹ and the number of repli-

cas for baseline measurement is recommended at $n = 20$.¹⁹² In the first approach, the electrical metric is measured in presence of different concentrations of analyte, and the resulting response is extrapolated towards lower concentrations. The LOD is then identified as the concentration value at which the extrapolation function intersects with the upper bound of the interval of confidence. For example, Chen *et al.*⁵⁰ used a linear fit to extract a correlation between the relative current change observed in time series and the logarithm of analyte concentration. From data taken in a range of 10 nM to 100 μM , they extrapolated this linear fit until reaching a signal-to-noise ratio of 3, and obtained an LOD value of 0.3 nM. More complex non-linear fits can be used as well for extrapolation, such as a Hill-Langmuir equation as used by Li *et al.*³⁹ The second approach consists in actually making measurements at decreasing concentrations, with replicas, until the observed change in the electrical metric is no longer statistically different from the interval of confidence. For instance, Cai *et al.*⁴³ measured the sensor response for concentrations down to 10 fM of complementary DNA. However, after assessing the noise level with a blank control test, the LOD was established at 100 fM, corresponding to a signal exceeding three times the background level. This underlines the importance of assessing the baseline value and its variance. In some specific cases, it is more relevant to use another threshold than the baseline signal to determine the LOD. For example, in Campos *et al.*,⁸¹ the LOD of perfectly-matched DNA is determined as the concentration sharing the same signal value as the highest signal obtained with single-mismatched DNA. In general, the LOD assessment method should be detailed in order to enable proper benchmarking between studies. In our literature survey (see ESI†), the LOD values were taken as reported, without adjustment for the determination method. Extrapolation methods tend to require less measurements, but the extracted LOD can be off the mark if analyte signal deviates from the extrapolation model at low concentrations. In this case the second type of approach is the most reliable as it ensures that



Another class of negative controls is to test the sensors against the targeted analyte, but without the appropriate probe, to ensure that the response is due to analyte:probe binding and not to non-specific adsorption of the analyte on the sensor. Several groups have reported on the non-specific interaction of their targeted analyte with pristine graphene without probes.^{78,78,121,121,125} A limitation of such experiments is that non-specific interactions of the analyte with the sensor surface are likely to be very different between pristine and probe-functionalized graphene. A strategy to better simulate the actual sensor surface is to prepare sensors with alternative probes having no affinity for the targeted analyte.^{42,49,55} For instance, Lerner *et al.*⁴⁹ functionalized the surface of control sensors with scFv fragments of anti-HER2 antibodies, unspecific to the target nalodextrone, instead of the specific MUR μ -receptor. Similarly, Hajian *et al.*⁵⁵ prepared control sensors by loading a non-complementary single-guide RNA sequence in the dCas9 protein (instead of the complementary sequence). Such approaches enable control experiments with a sensor interface very similar to the regular experiment.

Positive controls are meant to validate that the sensor generates a signal if the analyte is present in the sample. The most common approach is to prepare calibration samples containing a known concentration of the targeted analyte. Most studies look for a dependence with analyte concentration to demonstrate that the measured signal is indeed due to the analyte. In such assays, target molecules are most commonly diluted in blank saline buffer,^{42,88,90,117} sometimes with a calibrated mix of interfering species.^{53,55,83,91,118} These calibration assays are usually presented as a proof of concept for the sensors, and they are sometimes used as positive controls before assays on cell culture samples,^{51,56} clinical samples^{55,115,121,136,198} or other environmental samples,^{46,123} in which the concentration is either unknown or measured with another detection technique to compare results. For instance, Wang *et al.*¹²¹ were the first group to measure the concentration of lead ions in real blood samples with GFET sensors, using a calibration with positive controls in buffer. GFETs results were found in good agreement with measurements by ICP-MS, confirming the potential of GFET technology for medical applications. For large analytes, some studies use imaging strategies to confirm by visualization the immobilization of the analyte on the GFET surface. As examples, Chen *et al.*⁷⁸ performed fluorescence microscopy, scanning electron microscopy (SEM), and atomic force microscopy (AFM) to visualize the capture of *E. coli* bacterial cells onto the surface of functionalized GFET devices, and Xu *et al.*¹⁵⁸ used DNA probes labelled with Cy3 or Cy5 fluorophores to correlate the electrical response of GFETs with fluorescence measurements.

4.4. Response time

An important practical aspect of sensor performance is the test duration, *i.e.* the time required to obtain the result of an analysis. For a given sensor technology, estimating this metric is critical to identify potential applications and to determine how

the sensors will be packaged, deployed and used. GFETs are often praised as fast detection tools,^{53,54,120,200} however there is often a lack of clarity as to which steps of their operating protocol are included in this assessment. Specifically, the process of analyte detection or quantitation using GFETs usually require several steps: sample injection, incubation, washing, all repeated for a number of replica and controls. The duration of each of these steps can be informed by real-time measurements with time series. In particular, the response time, *i.e.* the time required for the signal to stabilize after injection of the sample, or after its washing away, can be extracted from time series. Independently of analyte binding kinetics, part of the response time comes from the basal response of the sensor to perturbations in the medium when injecting/washing the sample or changing applied electric potentials. It is possible to assess this contribution to the response time by changing abruptly the gate voltage and measuring the response of electrical metrics in time series.⁶¹

Response times reported for GFETs in the literature vary significantly between experiments, even for similar analytes. For ions, response times are usually small, from almost instantaneous^{51,122,123} to approximately 100 seconds.³⁹ For proteins, there is a wider variability: for example, Lei *et al.*¹⁷⁴ recorded signal reaching a plateau 10 s after insertion of the analyte brain natriuretic peptide, whereas Kim *et al.*⁴¹ assessed a response time to the prostate antigen PSA of approximately 10 minutes. For DNA hybridization, most studies focus on quantitation experiments using before/after measurements. Incubation times between the two are typically long to maximize hybridization density: from 30 minutes^{65,161} to many hours.⁴⁴ Outside that range, Hajian *et al.*⁵⁵ reported a detection of 1.7 fM in 15 minutes, due to the dCas9 system which actively improves the processing of DNA strands in the sample. The experimental process used to determine these incubation times is seldom described and likely to be based on trial and error, although a few real-time experiments have looked specifically at hybridization and denaturation kinetics.^{53,57} Some groups notice a significant variation of the response time with target concentration,^{53,57} such as observed by Xu *et al.*⁵³ in Fig. 2h. Others define the response time as the time required to reach current saturation in all tested concentrations, in order to determine an incubation time independent of target concentrations.^{41,42} Finally, continuous flow settings appear a promising solution to minimize incubation times, as they report faster response times than standard configurations based on injection followed by static incubation periods. With a 30 $\mu\text{L min}^{-1}$ flow, Stine *et al.*¹³⁴ reported a saturation of signal in less than 800 seconds for the hybridization of fully complementary ssDNA targets at 1 μM concentration. Xu *et al.*⁵³ obtained a stabilized hybridization signal in less than a minute for the same target concentration at 60 $\mu\text{L min}^{-1}$ flow.

4.5. Other considerations

Depending on which applications are targeted for the GFET biosensors, numerous practical issues are important to con-



sider in assessing their suitability and potential performance. Here, we merely raise some of the considerations that have been explored in the development of GFET sensor technology. Scalable production is an important aspect towards commercialization, in order to achieve competitive production rates and costs: scalable processes for GFET assembly have been developed, usually based on CVD synthesis techniques.^{65,201} Reusability, *i.e.* the possibility to make successive analyses on the same device, as well as shelf-life of the sensors are other important parameters in technology maturation, and have been tested in some recent studies. For example, reusability has been tested by Wu *et al.*¹⁹⁸ by performing successive binding–unbinding cycles with the target and measuring the evolution in the strength of signal, finding a conservation of signal of 94% when comparing the first to the last cycle. Some groups also made assessments of shelf-life by measuring the drift of current over time for multiple concentrations of target¹¹⁹ or by repeating experiments after storage time.⁶⁴ Reported shelf-life values vary from one week⁴⁹ to a few months.^{119,156} The use of flexible substrates is also an ongoing area of investigation for wearable or skin-implanted devices, for example to detect glucose levels in sweat directly on the skin¹⁵⁹ or for other health-monitoring purposes.²⁰² Silk fibroin,⁹¹ paper substrates^{92,202} or polyimide¹⁵⁹ have been successfully tested as flexible substrates.

5. Conclusions

Graphene field-effect transistors have demonstrated promising performance as bioanalytical sensors, including low limits of detection and fast response times in a miniature footprint. Their core feature is the use of graphene conductance as transducer, which provides high sensitivity to the capture of biomolecular species at its surface due to its monoatomic thinness. For the past decade, GFET sensors have been prototyped for a wide variety of biologically-relevant analytes: ions, small molecules, nucleic acids and proteins. We reviewed this literature (see ESI†) to discuss best practices in sensor assembly, experimental design and performance assessment, in particular towards the detection, quantitation and kinetic analysis of biomolecules. In sensor design, the type of graphene (exfoliation, CVD, rGO) does not appear as a dominant factor for performance: very low LODs have been reported for high- and low-quality graphene. Two more critical features are the configuration of the gate electrode and the assembly of the biorecognition interface: both would benefit from better modeling of their effect on graphene conductance transduction. In particular, the specifics of the surface chemistry (*e.g.* coverage, orientation, stability of immobilized probes, blocking species and captures analytes, and their respective interactions) are often not well known or controlled. On the other hand, limitations due to media screening appear well-understood and modeled by the Debye length, and strategies have been successfully proposed to increase the range of detection. The transduction of

analyte capture in electrical conductance can appear as a change in the density of charge carriers by doping (*i.e.* shift of the charge neutrality point), in the scattering processes (*i.e.* change in the transconductance), or a combination of them. Transfer curves, alone or combined with time series, are the most appropriate way of studying the physics of this interaction. Some interrogations remain about the coupling mechanism between analyte capture and graphene, as shown by the diverging CNP shift polarities reported for similar analytes. Time series of electrical current alone are not sufficient to interpret interactions mechanisms, but they can provide a robust empirical assessment of target presence or quantification, and they are essential for kinetic studies. Two-dimensional time series combining gate voltage and time sweeps provide both mechanistic and kinetic information in the same measurement. In all cases, in order to produce a reliable and reproducible experiment, intra- and inter-device variabilities need to be assessed and managed using sufficient replicas and appropriate controls. Finally, scalability and cost of fabrication, electronics and fluidics packaging for practical use with samples, as well as reproducibility and stability of the sensor response are important aspects to optimize in order to move the technology forward.

Conflicts of interest

There are no conflicts to declare.

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