Graphene-based bioelectrochemistry and bioelectronics: A concept for the future?

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GRAPHENE BASED BIOELECTROCHEMISTRY AND BIOELECTRONICS: A CONCEPT FOR THE FUTURE?

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Introduction

Clinical analysis benefits world-wide from a variety of diagnostic tests. These analytical tests should be fast and highly accurate to help in establishing a treatment protocol that is appropriate for the patient. The interest in the development of new clinical tests is not only driven by the demand to sense new analytes, but also to reduce costs, complexity and lengthy analysis times of current techniques. Among the myriad of possibilities available today, electrochemical and field-effect based biosensors are prominent players [1,2]. These analytical platforms require little and cheap instrumentation, can provide low detection limits once optimized, and be easily miniaturized, offering many of the desirable attributes for point-of-care tests. The best known example of an electrochemical based diagnostic device is the glucose biosensor, used widely in commercial glucometers and pH electrodes. Due to interest of patients as well as clinicians alike in these efficient, fast and simple to use sensing platforms, the number of biosensors developed in research laboratories integrating the market are gradually increasing.

A typical bioelectronics sensor contains two basic functional units: (i) an electrical transducer and (ii) a recognition element. The sensor selectivity is governed by surface-linked recognition elements (oligonucleotides, antibodies, enzymes, etc) as well as anti-fouling molecules (polyethylene glycol derivatives, serum molecules, etc) to limit non-specific signal, while the sensitivity is conferred by the transducer, converting the recognition event into a measureable electrical signal. One of the challenges in this field, concerns the sensitive and selective recording of biological recognition events in complex media such as human serum and salvia where picomolar (pM) and even lower detection limits for biological analytes are often requested. For advanced biosensors, the choice of the electrical transducer, the

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recognition element and the surface linking strategy, have to be considered simultaneously, making the construction of viable biosensing platforms of at great challenge. The attractive properties of graphene based nanomaterials have paved the way for the fabrication of a wide range of electrical and electrochemical based biosensors with improved analytical performance [3-6]. Different architectures are proposed to integrate graphene-based materials with a range of detection techniques implemented to record the biological recognition events. The materials science aspect has become one of the main research focus in the last years for graphene based bioelectronics platforms and the examples of the type of graphene materials used are almost countless (**Figure 1**).

This short review provides insights into the various benefits of graphene based bioelectrical and bioelectrochemical sensors. Situated at the interface of materials science, chemistry and the life sciences, graphene based electronics and electrochemistry offer a broad palette of opportunities for researchers and clinicians for targeted theranostics in biology and medicine.

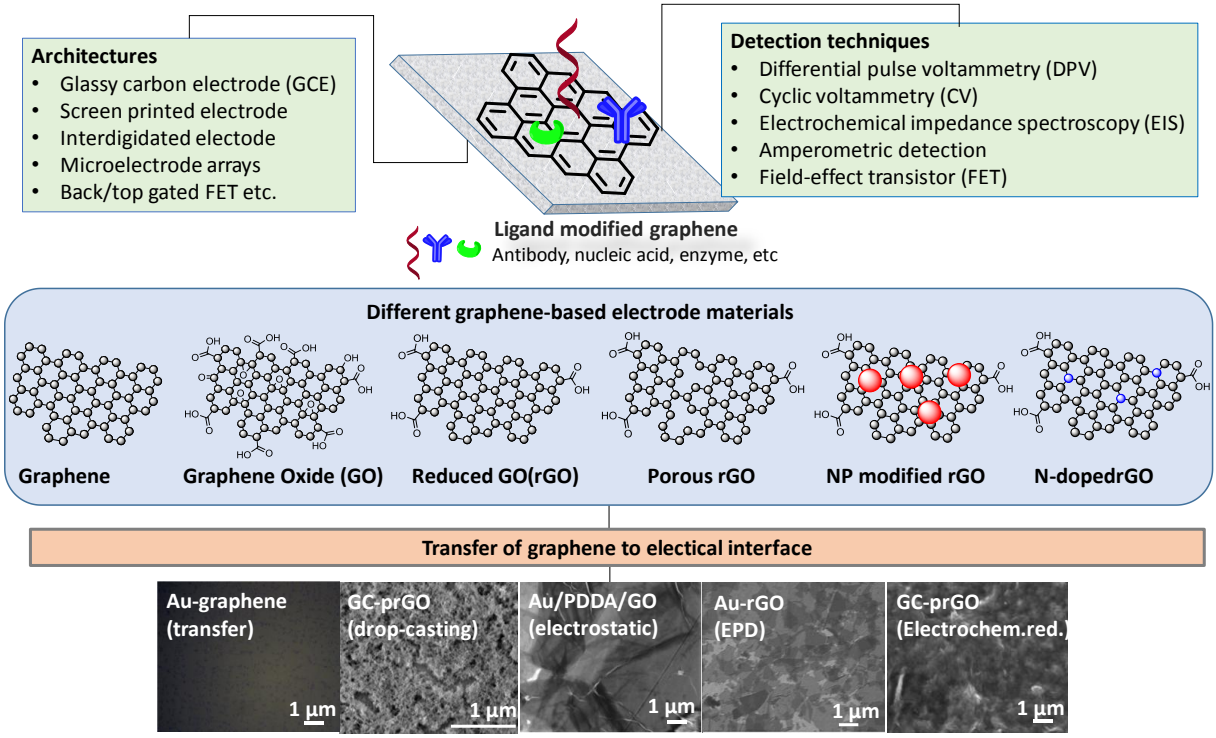


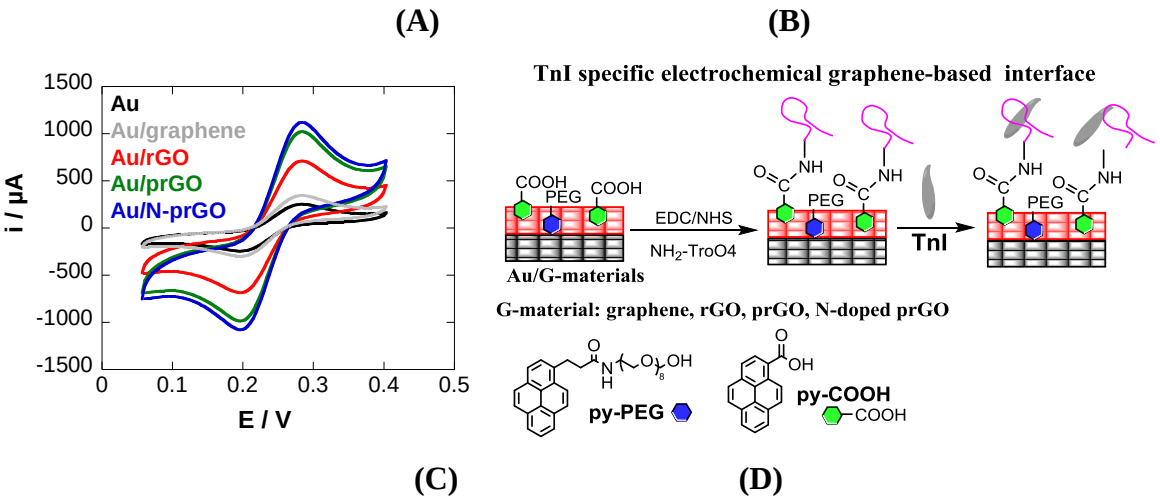
Figure 1: Different components for the construction and use of graphene based bioelectronics and bioelectrochemical sensors.

Electrochemical based sensing: Aspects about electron transfer on different graphene modified interfaces

The underlying principle of an electrochemical sensor is to convert a biological binding event into a measurable electrical signal. This approach is represented by electron transfer between the transducer, being graphene coated electrical interfaces in our case, and an electroactive species, which can be either the molecule to be analyzed or a species, which electrochemical signal correlates with the presence of the target analyte. Aspects governing electron transfer on graphene coated electrodes become of importance to obtain large output signals, which will allow reaching the low detection limits needed when analyzing clinical samples. Given the enormous variety of graphene-based materials (**Figure 1**), the question of where electron transfer occurs and what are the parameters influencing electron transfer rate are often difficult to answer, and mostly neglected in the construction of bio-electrochemical sensing interfaces. Impressive electrocatalytic performance was obtained on multi-layered graphene flakes coated Si wafers in response to biomolecules such as dopamine, ascorbic acid, attributed to the large area of edge planes that are available on such an interface to allow rapid heterogeneous electron transfer [7]. Simple solution processable reduced graphene oxide (rGO), which enables large scale production, results in a graphene material with structural defects due to the presence of different oxygen functional groups, which are never completely removed. While these properties might impact the electrical readout, these defects are actually beneficial to achieve fast heterogeneous electron transfer and promote the possibility for covalent immobilization of surface ligands to target specific sensing applications [5,8]. Kamat and co-workers have lately looked into the aspect of controlled electron capture, transport and discharge of rGO modified glassy carbon electrodes (GCE), showing that cooperative effects of two redox couples (in this case ferrocene and methyl viologen) on a rGO platform can increase the sensitivity of electrochemical detection, something still not exploited for sensing, but opening up the possibility of detection at low levels [9].

The question is ultimately whether single-layer two-dimensional graphene, reduced graphene oxide (rGO), porous rGO, doped and functionalised rGO derivatives exhibit more rapid electron transfer over other electrodes and highly ordered pyrolytic graphite (HOPG). Comparison is a very difficult task as the way the graphene materials are coated onto the electrical interface will influence strongly the electrochemical behaviour. We opted on highlighting the complex issue by recording cyclic voltammetric profiles in ferrocyanide/KCl solutions of a gold electrode modified with CVD graphene [10], and by drop casting different suspensions of rGO (1 mg mL^{-1} in water) (**Figure 2A)** [11-14]. Deposition of CVD graphene results in a slight increase in the voltammetry peak height; the largest increase was achieved

on N-doped porous reduced graphene oxide, with the peak-to-peak potential separation remaining unchanged. The increase in the electron transfer rate on chemically transferred CVD graphene nanosheets is mostly due to the enhancement of the surface area as in fact a single layer is not completely flat and has a tendency to fold, buckle and corrugate. The presence of oxygen-related edge and plane functions as is the case in rGO based materials together with the increased active surface area results in superior electron transfer rates, surpassing those of gold and gold/CVD graphene [15]. The intrinsic chemical doping with heteroatoms is an effective way for modulating and tailoring the physico-chemical properties of graphene. Substitution with electron-rich atoms, such as nitrogen, which is able to form strong valence bonds with carbon atoms, can lead to an increase in free charge carriers in the graphene's aromatic ring network and therefore an enhanced conductivity. As seen in **Figure 2A**, porous nitrogen-doped graphene nanostructures lead to a further increase in the electrochemical electroactivity; this underlines the general trend that the sample with the highest defect density displays the fastest electron transfer kinetics [8,15]. This highlights that electron transfer rates depend strongly on the type, morphology, as well as on the film thickness of the graphene coatings, where each parameter acts differently on the electrochemical activity of graphene. The materials science part of graphene and its derivatives is thus of fundamental importance to overcome the ambiguity about the electrochemical properties of graphene.



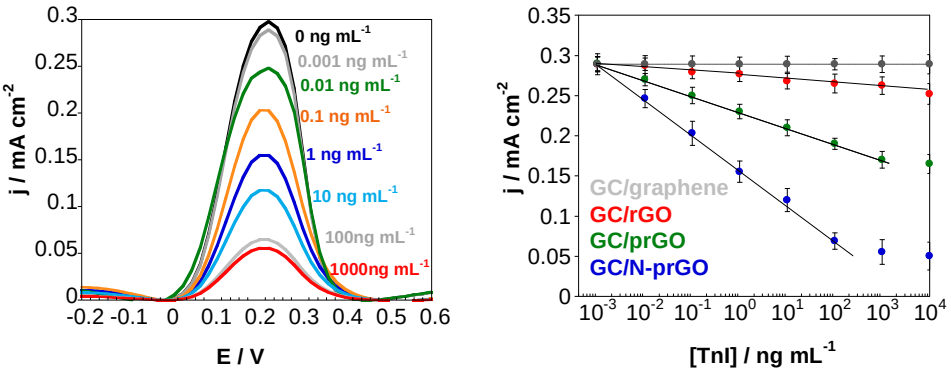


Figure 2: (A) Current *vs.* potential plots recorded by cyclic voltammetry on Au (black), Au/graphene (chemically transferred of CVD grown graphene); Au/rGO (red), Au/prGO (blue) and Au/N-doped prGO using $[Fe(CN)_6]^{4-}$ (10 mM)/PBS (0.1 M), scan rate = 100 mV s^{-1}; (B) Schematic presentation of the construction of an immunosensor for TnI; (C) Change in differential pulse voltammogram signal upon addition of TnI on N-doped pRGO modified electrode; (D) Calibration curve for TnI on different interfaces.

To further demonstrate how the electron transfer kinetics can affect the sensitivity and linear range of an electrochemical sensor, a toponin I (TnI) specific sensor was constructed lately upon covalent integration of aptamer Tro4 (**Figure 2B)**. Addition of TnI results in a decrease of the differential pulse voltammetry (DVP) signal of ferrocyanide, an inner-sphere redox couple sensitive to surface functions (**Figure 2C)**, which scales linearly with the TnI concentration down to 1 pg mL^{-1} over a wide linear range of 5 orders of magnitude between 1 pg mL^{-1} and 100 ng mL^{-1} with a slope of 43 µA cm^{-2}/decade (r^2=0.9992) (**Figure 2D)**.

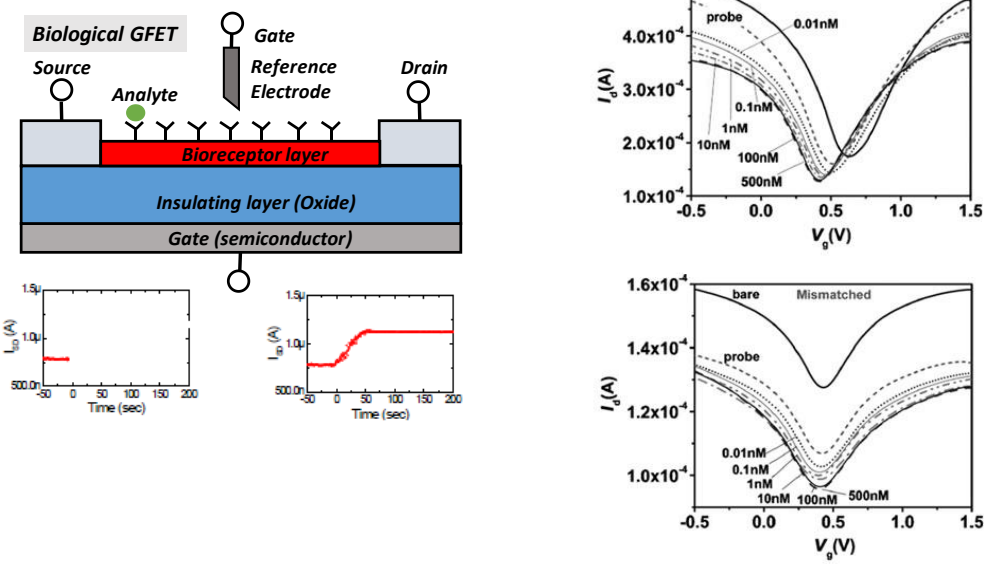
Graphene based biological field-effect transistors (GFET)

Utilizing a 2D channel material such as graphene in back or liquid gated bioFETs has several advantages over classical FET technology. For most semiconductor-based transistor sensors, local electric field changes at the channel surface have little effect deeper in the device channel, limiting the response sensitivity. With a GFET (**Figure 3A**), the graphene channel is only one atom thick, meaning the entire channel is effectively on the surface and directly exposed to the environment. Any molecule attached to the surface of the channel impacts electronic transfer through the entire depth of the device and can be sensed. When target molecules bind to the receptors on the graphene surface, the redistribution of electronic charge generates a change in the electric field across the GFET channel region, which changes the electronic conductivity in the channel and the overall device response as seen in the

transfer curve of a liquid-gated GFET for the analysis of DNA hybridization events (**Figure 3B**). Most importantly, for sensing applications using GFETs, the Dirac point is sensitive to the presence of immobilized DNA probes as well as to the hybridization of complementary target DNA [16].

The main concern with biological GFETs remains the effect of ionic, also called double-layer screening (**Figure 3C**). This screening effect is dependent on the distance between the FET sensing surface and the point of observation and is characterized by the Debye screening length (λ_D), defined by the distance where the electrical signal decays to 1/e of its original value. Unless very diluted solutions or salt free solutions are considered, typical screening lengths are in the order of 1 nm with stronger screening effects for larger molecules. The use of small molecular sized ligands (antigen binding fragments, aptamers) are thus currently reviling classical FET based immunosensors (**Figure 3D**) [17].

(A) **(B)**



(C) **(D)**

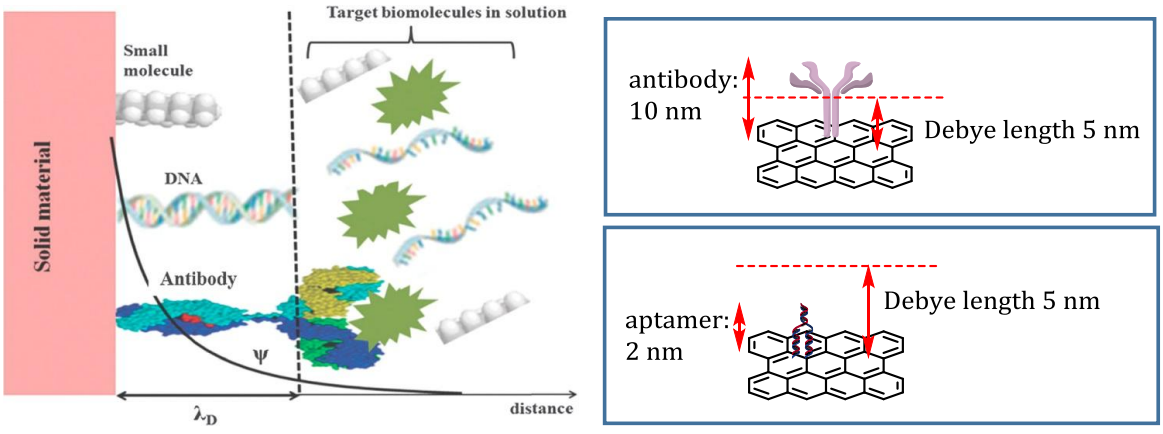


Figure 3: (**A**) Image of a liquid-gated GFET together with change in drain-source current upon a binding event; (**B**) Transfer characteristics of a GFET before adding DNA, after immobilization of probe DNA, and after reaction with (b) complementary or (c) one-base mismatched DNA molecules with the concentration ranging from 0.01 to 500 nM. (Reproduced with permission from Ref. [16]; (**C**) Electrical double-layer length in the presence of different targets (dimensions are not scaled) (Reprinted with permission from Ref. [18]), (**D**) Influence of ligand size on FET based sensing.

OPINION ON THE FUTURE OF GRAPHENE BASED ELECTRICAL AND ELECTRIOCHEMICAL SENSING

The examples of graphene based electrochemical and electrical based biosensors are countless and vary not only in the way the graphene based interface is used, but also in the manner electrical or electrochemical read out is achieved (**Table 1).**

Table 1: Selected recent examples of bioelectronic and bioelectrochemical graphene based sensors.

analyte	electrode	method	Linear range	LoD	comments	Ref
CEA	G	FET		0.5 pM	PASE+anti-CEA	[19]
micro-RNA	rGO	FET		10 fM	Au-NPs modified PNA	[20]
SNP	G	FET	100 nM-100 µM	100 nM	+1-pyCCOH	[21]
nucleic acid	Thiofluoro-graphene	EIS			nucleic acid adsorption	[22]
Interleukin-8	rGO	DVP	500 fg mL^{-1}-4 ng mL^{-1}	72.7 pg mL^{-1}	Au NPs+anti-IL8	[23]
PSA	rGO	DPV	90 ng mL^{-1}-0.1 pg mL^{-1}	10 fg mL^{-1}	+aptamer	[24]
lysozyme	prGO	DVP	0.05-7.5 µM	50 nM	Direct oxidation	[12]
FAP	rGO (EPD)	DVP	1-100 pM	1 pM	folic acid modified	[13]
gliadin	prGO	DVP	1.2-34 ng ml^{-1}	1.2 ng ml^{-1}	PA+anti-gliadin	[11]
PSA	NH_2-graphene	EIS	2 pg ml^{-1}-2 µg ml^{-1}	0.46 pg ml^{-1}	+ anti-PSA and Au NP amplification	[25]
thrombin	Laser-scribed G-electrode	DVP	1 pM-0.1 nM	1 pM	PA+thrombin aptamer	[26]
VEGF165	rGO	SWP	10 fg ml^{-1}- 1 ng ml^{-1}	8 fg mL^{-1}	Cucurbituril and N_3 modified rGO +alkynyl-DNA+aptamer	[27]
Dopamine	rGO/PEI (EPD)	DVP		50 nM	microsystem	[28]
Folic acid	rGO/MoS_2	DVP	0.01-100 µM	10 nM	Direct oxidation	[29]
glucose	rGO/N-doped prGO/CuO	CA	0.25µM-6 mM	0.25 µM	EPD	[14]
H_2O_2	rGO/Pt NPs	CA	0.2 nM-3 µM	20 nM	chitosan-ferrocene carboxylic acid	[30]
H_2O_2	rGO-TiO_2	CA	0.1-360 µM	10 nM	+hemoglobin	[31]

CA: chronoamperometry; CEA: carcinoembryonic antigen; DPV: differential pulse voltammetry; ESI: Electrochemical impedance spectroscopy; EPD: electrophoretic deposition; FAB: folic acid protein; G: pristine graphene, PA: 1-pyrenebutyric acid, PASE: 1-pyrenebutanoic acid succinimidyl ester, prGO: porous reduced graphene oxide, PSA: prostate specific-antigen; rGO: reduced graphene oxide; SNP: single nucleotide polymorphism; SWP: single sweep potential.

While advances in graphene materials have shown to be highly crucial for improving electrical and electrochemical biosensor characteristics, different challenges are still to overcome. There is still an urgent need for moving beyond research by developing new concepts for achieving even better sensitivity and selectivity, in order to bring some of the current sensors into real biomedical applications. Even though a large amount of graphene based sensors reported in the literature exhibit good sensing characteristics, the performance in real biological samples, where next to a variety of other proteins high salt concentrations are present, is often not considered and information is largely lacking. As non-specific interactions of these proteins are of primary concern on graphene based interfaces, this lack of information is one of the two most crucial limiting factors for current commercialization. While some efforts have been made here by the integration of polyethylene modified pyrene ligands as well as blocking with human serum itself, more efforts have to be put into this direction to optimize the selectivity of the sensor when used in complex biological media. The other limiting factor for commercialisation is the current limitation of large-scale reproducibility of the fabrication of graphene biosensor interfaces.

In the case of bioelectronics, the overbearing sensitivity-limiting factor from the Debye screening length must be mitigated to allow for sensing in high ionic strength solutions such as serum, saliva, etc. A detailed understanding of aptamer/analyte affinity interaction and strength becomes a fundamental issue here to ensure promising bioelectronics detection of various proteins. This is also important to convince industry to change from the well-known and highly specific antibody strategies to less selective aptamer technology.

CONCLUSION AND OUTLOOK

Graphene and its derivatives prove to be a rich source for the construction and use of bioelectronics and bioelectrochemical sensors. Routinely, such sensors achieve a picomolar detection limit, with some even reaching the low femtomolar concentration range. The possibility that a large range of different detection methods can be employed with graphene based sensors is of high advantage, as depending on the looked after final application, sensor size and read out can be customised at will. The collaboration between materials scientists,

chemists, physicists as well as engineers and medical personal is of fundamental importance to drive this field further and to propose graphene based biosensors as point-of-care alternatives for patients. The success of any new biosensor material lies in addition on its reproducibility and possible industrial scale production. The emergence of a number of companies providing mono- and bilayered graphene nanosheets on several interfaces, graphene oxide (GO), reduced graphene oxide (rGO) and even modified matrixes has been an additional motivation for using graphene for biosensor applications. When it comes to *in vivo* application of some of the sensing concepts, graphene based biosensors are still in their infancy. Toxicity and biocompatibility issues still need to be addressed carefully to avoid any undesired secondary health effects. Current *in vivo* and *in vitro* assessments of the biostability of the sensors are encouraging and promising for further technological transfer. Finally, beside various integration techniques insuring highly sensitive sensing, the prevalence of the current mobile area has introduced the emergence of portable and smartphone based analytical quantification read outs using wireless technology. It will be exciting to witness the futures of these wireless-based sensing devices which will finally make real personalized diagnostic possible.

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Reference and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

•Paper of special interest

••Paper of outstanding interest

1. Bunyakul N, Baeumner AJ: **Combining Electrochemical Sensors with Miniaturized Sample Preparation for Rapid Detection in Clinical Samples**. *Sensors* 2015, **15**:547-564
2. * Kaisti M: **Detection principles of biological and chemical FET sensors**. *Biosens. Bioelectron.* 2017, **98**:437-448.

3. * Ibau C, Md Arhsad MK, Gopinath SCB: **Current advances and future visions on bioelectronic immunosensing for prostate-specific antigen**. *Biosens. Bioelectron.* 2017, **98**:267-284.

Important as a recent comprehensive overview of immunosensors for PSA

4. Pandikumar A, How GTS, See TP, Omar FS, Jayabal S, Kamali KZ, Yusoff N, Amil A, Ramaraj R, John SA, et al.: **Graphene and its nanocomposite material based electrochemical sensor platform for dopamine** *RS Adv.* 2014, **4**:63296-63323
5. * Ambrosi A, Chua CK, Latiff NM, Loo AH, Wong CHA, Eng AYS, Bonannia A, Pumera M: **Graphene and its electrochemistry – an update** *Chem. Soc. Rev.* 2016, **45**:2458-2493.

Important as a recent comprehensive overview of pf graphene for electrochemical applications

6. Turcheniuk T, Boukherroub R, Szunerits S: **Gold–graphene nanocomposites for sensing and biomedical applications**. *J. Mater. Chem. B* 2015, **3**:4301-4324
7. Shang NG, Papakonstantiou P, McMullan M, Chu M, Stamnoulis A, Potenza A, Dhesi SS, Marchetto H: **Catalyst-Free Efficient Growth, Orientation and Biosensing Properties of Multilayer Graphene Nanoflake Films with Sharp Edge Planes†**. *Adv. Func. Mater.* 2008, **18**:3506-3514.
8. Zhou M, Zhai M, Dong S: **Electrochemical Sensing and Biosensing Platform Based on Chemically Reduced Graphene Oxide**. *Anal. Chem.* 2009, **81**:5603-5613.
9. ** Bridewell VL, Karwacki CJ, Kamat PV: **Electrocatalytic Sensing with Reduced Graphene Oxide: Electron Shuttling between Redox Couples Anchored on a 2-D Surface**. *ACS Sens.* 2016, **1**:1203-1207.

This paper is unique in demonstrating the importance of elecron shutteling on rGO

10. Zagorodko O, Spadavecchia J, Yanguas Serrano A, Pesquer A, Zurutuza A, Boukherroub R , Sabine S: **Highly Sensitive Detection of DNA Hybridization on Commercialized Graphene-Coated Surface Plasmon Resonance Interfaces**. *Anal. Chem.*, 2014, **86**:11211–11216.
11. Chekin F, Sing SS, Vasilescu A, Dhavale V, Kurungot S, Boukherroub R, Szunerits S: **Reduced Graphene Oxide Modified Electrodes for Sensitive Sensing of Gliadin in Food Samples**. *ACS Sens.* 2016, **1**:1462-1470.
12. Vasilescu A, Boulahneche S, Chekin F, Gáspár S, Medjram MS, Diagne AA, Singh SK, Kurungot S, Boukherroub R, Szunerits S: **Porous reduced graphene oxide modified electrodes for the analysis of protein aggregation. Part 1: Lysozyme aggregation at pH 2 and 7.4**. *Electrochim. Acta* 2017, **254**:375-383.
13.* He L, Wang Q, Mandler D, Li M, Boukherroub R, Szunerits S: **Detection of folic acid protein in human serum using reduced graphene oxide electrodes modified by folic-acid**. *Biosens. Bioelectron.* 2016, **75**:389-395.

Important as it is an amplificaiton less example for folic acid protein detection in serum

14. Maaoui H, Singh SK, Teodorescu F, Coffinier Y, Barras A, Chtourou R, Kurungot S, Szunerits S, Boukherroub R: **Copper oxide supported on three-dimensional ammonia-doped porous reduced graphene oxide prepared through electrophoretic deposition for non-enzymatic glucose sensing**. *Electrochim. Acta* 2017, **224**:346-354.
15. Lim CX, Hoh HY, Ang PK, Loh KP: **Direct Voltammetric Detection of DNA and pH Sensing on Epitaxial Graphene: An Insight into the Role of Oxygenated Defects**. *Anal. Chem.* 2010, **82**:7387-7393.
16. Dong X, Shi Y, Huang W, Chen P, Li LJ: **Electrical detection of DNA hybridization with single-base specificity using transistors based on CVD-grown graphene sheets**. *Advanced Materials* 2010, **22**:1649-1653.

17. Cheng S, Hotani K, Hideshima S, Kuroiwa S, Nakanishi T, Hashimoto M, Mori Y, Osaka T: **Field Effect Transistor Biosensor Using Antigen Binding Fragment for Detecting Tumor Marker in Human Serum**. *Materials* 2014, **7**:2490-2500.
18. Huang W, Diallo AK, Dailey JL, Besar K, Katz HE: **Electrochemical processes and mechanistic aspects of field-effect sensors for biomolecules**. *J. Mater. Chem. C* 2015, **3**:6445-6470.
19. Zhou L, Mao H, Wu C, Tang L, Wu Z, Sun H, Zhang H, Zhou H, Jia C, Jin Q, et al.: **Label-free graphene biosensor targeting cancer molecules based on non-covalent modification.** *Biosens. Bioelectron.* 2017, **87**:701-707.
20. Cai B, Huang L, Zhang H, Sun Z, Zhang Z, Zhang GJ: **Gold nanopaticles-decorated graphene field-effect transistor biosensor for femtomolar MicroRNA detection**. *Biosens. Bioelectron.* 2015, **74**:329-334
21. ** Hwang MT, Landonb PB, Lee J, Choi D, Mo AH, Glinsky G, Ratnesh Lal R: **Highly specific SNP detection using 2D graphene electronics and DNA strand displacement**. *Proc. Natl. Acad. Sci. USA* 2016, **113**:7088-7093.

Unique paper reporting on the use of CVD graphene in an electronci platform for SNP detection with great sensitivity

22.** Urbanová V, Holá K, Bourlinos AB, Čépe K, Ambrosi A, Loo AH, Pumera M, Otyepka M, Zbořil R: **Thiofluorographene–Hydrophilic Graphene Derivative with Semiconducting and Genosensing Properties**. *Adv. Mater.* 2015, **27**:2305-2310.

Unique paper reporting on the importance of surface functinonality of graphene for sensing

23. Verma S, Singh A, Shukla A, Kaswan J, Arora K, Ramirez-Vick J, Singh P, Singh SP: **Anti-IL8/AuNPs-rGO/ITO as an Immunosensing Platform for Noninvasive Electrochemical Detection of Oral Cancer**. *ACS Appl. Mater. Interfaces*, 2014, **9**:27462-27474.
24. Kovasi B, Salimi A, Hallaj R, Moradi F: **Ultrasensitive electrochemical immunosensor for PSA biomarker detection in prostate cancer cells using gold nanoparticles/PAMAM dendrimer loaded with enzyme linked aptamer as integrated triple signal amplification strategy.** *Biosens. Bioelectron.* 2015, **74**:915-923.
25. Yan M, Zang D, Ge S, Ge L, Yu J: **A disposable electrochemical immunosensor based on carbon screen-printed electrodes for the detection of prostate specific antigen**. *Biosens. Bioelectron.* 2012, **38**:355.
26. Fenzl C, Nayak P, Hirsch T, Wolfbeis OS, Alshareef HN, Baeumner AJ: **Laser-Scribed Graphene Electrodes for Aptamer-Based Biosensing**. *ACS Sens.* 2017, **2**:616-620.
27.* Wei T, Dong T, Xiang H, Liu Y, Dia Z: **Cucurbituril and Azide Cofunctionalized Graphene Oxide for Ultrasensitive Electro-Click Biosensing.** *Anal. Chem.* 2017, **acs.analchem.7b03068.**

Important as it focuses on elctro-click onn graphene oxide for sensing

28. Kahlouche K, Jijie R, Hosu I, Barras A, Gharbi T, Yahiaoui R, Herlem G, Ferhat M, Szunerits S, Boukherroub R: **Controlled modification of electrochemical microsystems with polyethylenimine/reduced graphene oxide using electrophoretic deposition: Sensing of dopamine levels in meat samples**. *Talanta* 2018, **178**:432-440.
29. Chekin F, Teodorescu F, Coffinier Y, Pan G-H, Barras A, Boukherroub R, Szunerits S: **MoS_2/reduced graphene oxide as active hybrid material for the electrochemical detection of folic acid in human serum**. *Biosens. Bioelectron.* 2016, **85**:807-813.
30. Bai Z, Li G, Liang J, u J, Zhang Y, Chen H, Huang Y, Sui W, Zhao Y: **Non-enzymatic electrochemical biosensor based on Pt NPs/RGO-CS-Fc nano-hybrids for the detection of hydrogen peroxide in living cells.** *Biosens. Bioelectron.* 2016, **82**:185-194.

31. Liu H, Guo K, Duan C, Dong X, Gao J: **Hallow TiO_2 modified reduced graphehe noxide miscrosphere encasulating hemoglobin for a medator-free biosensor**. *Biosens. Bioelectron.* 2017, **87**:473-479.