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# Recent progress in the design of G-quadruplex based electrochemical aptasensors

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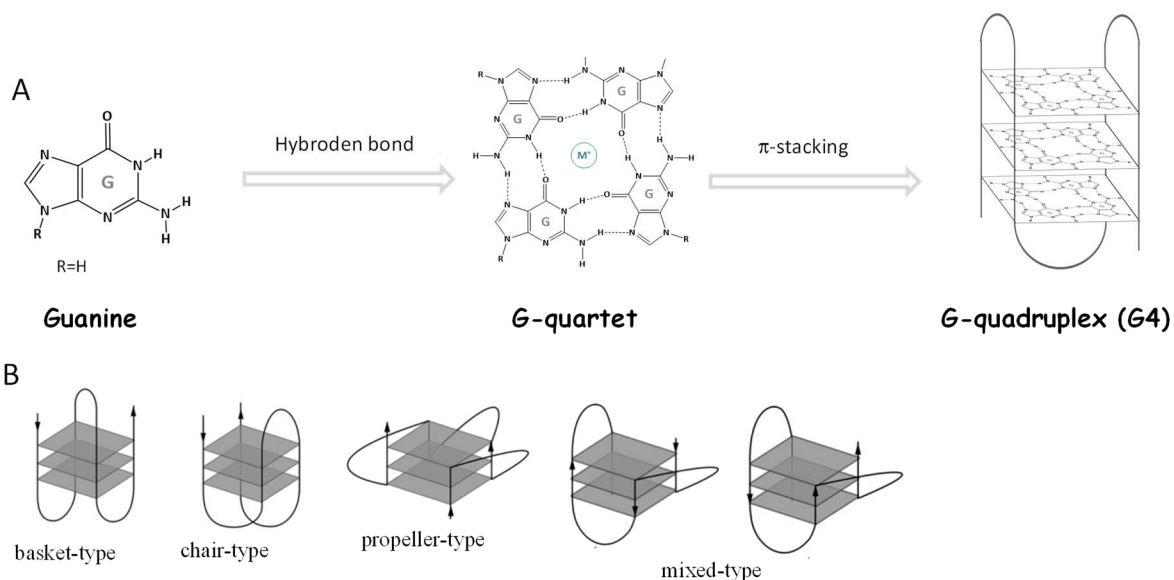
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### Abstract

Aptamer based electrochemical sensors are now developed for the detection of a wide variety of analytes including ions, low molecular weight molecules, proteins and living cells. An aptamer-based sensor is an analytical device whose the bio-sensing element (*i.e.* the aptamer) is immobilized on a transducer surface. Aptasensors have attracted great attention because of their high selectivity, sensitivity and stability, they could be miniaturized and are of low production cost and offer extraordinary flexibility in the design of their assemblies. This review will emphasize recent developments of aptasensors using aptamers able to adopt the particular G-quadruplex (G4) conformations, which are secondary DNA structures formed from guanine-rich sequences. Indeed, G4 exhibits notable recognition properties inherent to their particular structuration.

**Keywords:** Aptasensor, G-quadruplex, electrochemistry, aptamer

Aptasensors, for aptamers based biosensors, have demonstrated their usefulness for the detection of chemical, biochemical or biological targets in real matrices [1,2]. Aptamers consist of single-stranded oligonucleotides, DNA or RNA with length from 25 to hundred nucleobases, and are selected *in vitro* from a combinatorial library using the systematic evolution of ligands by exponential enrichment (SELEX) process. Selected aptamers usually show high specificity and selectivity for their target with high affinity (dissociation constant  $K_D$  within the nanomolar to picomolar range). By comparison with antibodies, they present advantageous properties such as their thermal and chemical stabilities, their easy chemical synthetic accessibility and thus multiple functional groups (*e.g.*, electrochemical or fluorescent labels, surface grafting functions) can be easily and regioselectively introduced to afford them additional properties [1]. An interesting feature of aptamers is that they can change their conformation upon binding to the target into secondary 3D-structures including loop [3], G-quadruplex (G4) [4] or kissing hairpin [5] which can be exploited for transduction [6–8]. G4 structures are formed from the folding of G-rich sequences and consist of stacked tetrads of Hoogsteen hydrogen-bonded guanine bases connected by various loop-forming sequences (Fig. 1A). Due to their rich structural polymorphism (*i.e.* parallel, antiparallel and hybrid topologies, Fig. 1B) they have shown an increasing interest for the design of aptasensors. A wide variety of aptamers could fold into G4 structures upon binding to their target. In this context, the detection of large biological components such as proteins *e.g.* thrombin [9], growth factors [10], protein tyrosine kinase-7 [11], insulin [12], cardiac troponin I [13], some cancer biomarkers [14], carcino embryonic antigen [15] as well as exosomes [16] and circulating tumor cells [17] have been reported. G4-based aptasensors also allow the detection of low molecular weight compounds including 8-hydroxy-2'-deoxyguanosine [18], antibiotics [19], bisphenol A [20], mycotoxin [21], and metal ions  $K^+$  [22] and more specifically heavy metals [23] such as As(III) [24],  $Hg^{2+}$  and  $Pb^{2+}$  [25,26]. Herein, we aim to provide an overview of the recent development of biosensors based on G4 DNA.



**Figure 1.** A/Chemical structure of guanine and scheme of the formation of G quartet and of a G-quadruplex B/ various G-quadruplex topologies.

An electrochemical aptasensor is an analytical device integrating the aptamer as bioreceptor in close contact with an electrochemical transducer which convert the target-aptamer interaction into an electrochemical signal. The sensing element (*i.e.* the aptamer probe) is immobilized directly on the electrode substrate used as inert conductive platforms (mainly gold, glassy carbon or platinum) or onto nanostructured materials which are subsequently grafted on the electrode surface thus allowing the enhancement of the aptamer surface density. The associated nanomaterials are in most of the cases carbon nanotubes [27], graphene-based nanostructures [28–30], metal and metal oxide nanoparticles [31], metal-organic framework [25], conductive polymers [9,32,33] or a combination of them [21]. Such nanomaterials could contribute to the signal amplification due to their electrocatalytic properties which provide the increase of the electron transfer rates. Methods of immobilization include  $\pi$ - $\pi$  interactions mainly onto graphene-based nanostructures [34], physisorption onto gold surface *via* a terminal adenine nucleotides track (polyA) incorporated into single stranded DNA (ssDNA) [35,36] or through the self-assembly of thiolated DNA onto gold surfaces (followed by a backfilling with a small thiol molecule [37]), covalent bonding such as amine coupling [9] or avidin-biotin affinity interactions [38,39]. The immobilization method impacts the orientation and the accessibility of the sensing aptamer towards the target and the stability of the support material as well as the minimization of non-specific adsorption of the target.

The analytical performances of aptasensors depend both on the proper affinity of the selected aptamer for its target and on the electrochemical method which plays a crucial role in the sensitivity of the detection. The electrochemical transduction methods used for aptasensors could be categorized in potentiometry, voltammetry (amperometry) [40], electrochemical impedance spectroscopy (EIS) [41], field effect transistor (FET) [22] and electroluminescence [25]. Voltammetry including Square Wave Voltammetry (SWV) [42], Differential Pulse Voltammetry (DPV) [16], Cyclic Voltammetry (CV)[43] and Alternating Current Voltammetry (ACV) [12] represents the mainly used transduction method. The objective of this short review is to highlight the recent developments of G4-based electrochemical aptasensors for the detection of various target analytes. Instead of being exhaustive we will focus on recent selected publications of outstanding significance, we will in particular discuss about the sensing mechanism involved in the detection process.

A simple and effective assay format involves a single aptamer with a detection mode based on the modification of a single-stranded nucleic acid structure into a G4 conformation upon the analyte binding (Fig. 2) either onto the functionalized surface [12,22,35,41,44] (Fig. 2A and B) or in the solution [42,45] (Fig. 2C) following by an electrochemical detection.

**Table 1.** Properties of G4-based electrochemical aptasensors described in this review.

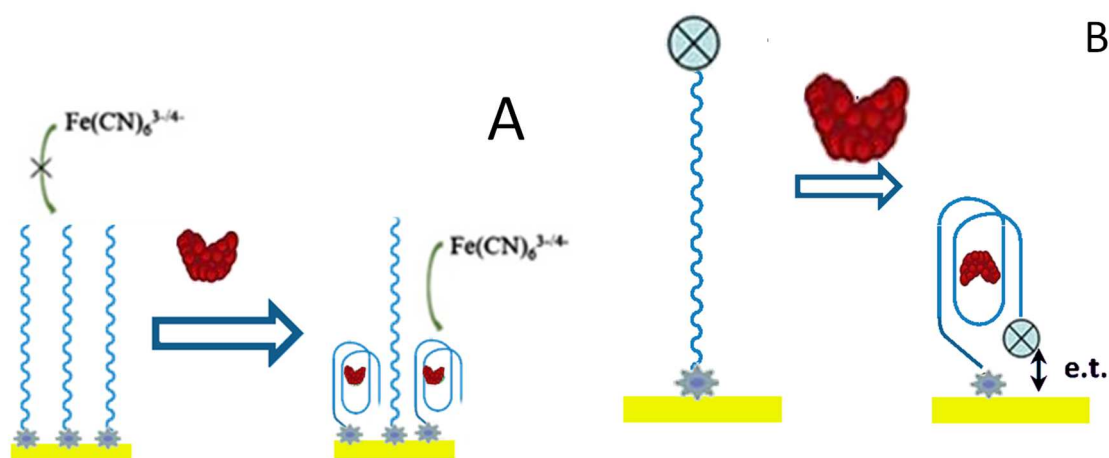
Entry	Analyte	Electrochemical technique	Linear range/dynamic range	LOD	Ref.
1	K <sup>+</sup>	FET	0.1pM - 100 nM	58 fM	[22]

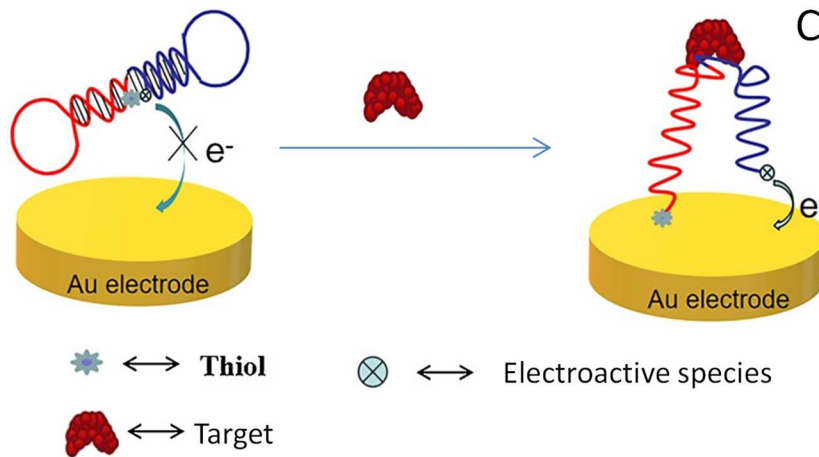
2	VEGF <sub>165</sub>	EIS	0.026 - 31.4 fM	0.017 fM	[41]
3	Insulin	SWV ACV	0.02 - 5 μM	20 nM	[12]
4	K <sup>+</sup>	SWV	10 nM - 100 μM	4.5 nM	[42]
5	Thrombin	DPV	1.2 pM - 12 nM	0.8 pM	[45]
6	Thrombin	DPV	1 pM to 10 nM	0.64 pM	[9]
7	Mycotoxins (OTA and AFB1)	ACV	0.03-10.0 ng mL <sup>-1</sup> for OTA and 0.01-3.0 ng mL <sup>-1</sup> for AFB1	0.0043 ng mL <sup>-1</sup>	[37]
8	Pb <sup>2+</sup>	CV	0.01 – 1000 nM	0.008 nM	[43]
9	OTA	DPV	0.001 – 0.5 ng mL <sup>-1</sup>	0.26 pg mL <sup>-1</sup>	[21]
10	Exosomes	DPV	4.8 × 10 <sup>3</sup> to 4.8 × 10 <sup>6</sup> Units mL <sup>-1</sup>	9.54 10 <sup>2</sup> Units mL <sup>-1</sup>	[16]
11	VEGF <sub>165</sub>	PEC	100 fM – 10 pM	30 fM	[46]
12	Hg <sup>2+</sup> , Pb <sup>2+</sup>	ECL	10 pM – 0.1 μM (Hg <sup>2+</sup> ) 100 pM – 1 μM (Pb <sup>2+</sup> )	4.1 pM (Hg <sup>2+</sup> ) 37 pM (Pb <sup>2+</sup> )	[25]

Yuan *et al* [22] proposed a sensitive-enhanced potassium ion (K<sup>+</sup>) selective detection using a graphene FET biosensor with the immobilization of the aptamer G4 forming sequence on a graphene-based conducting channel (Table 1 – Entry 1). Feng *et al.* [41] reported an impedimetric aptasensor based on a label-free gold line-pad-line electrode for the determination of vascular endothelial growth factor (VEGF<sub>165</sub>), an important biomarker for breast and lung cancers. Upon addition of VEGF<sub>165</sub>, a large target-induced conformational change of the specific aptamer anti-VEGF<sub>165</sub> into G4 structure was generated and caused an increase of the impedance magnitude of the electrochemical device (Table 1-entry 2).

Wu *et al.* have developed an electrochemical aptasensor for the detection of insulin [12] (Table 1 – Entry 3). The thiolated aptamer was labeled with methylene blue (MB) and immobilized on gold surface. The insulin binding induces a steric hindrance which reduces the efficiency of the electron transfer between the redox probe and the electrode surface and triggers a decrease of the electrochemical signals (ACV and SWV). Such so-called signal-off response (corresponding to a decrease of the electrochemical response compared to the initial signal without the analyte) offered a high sensitivity.

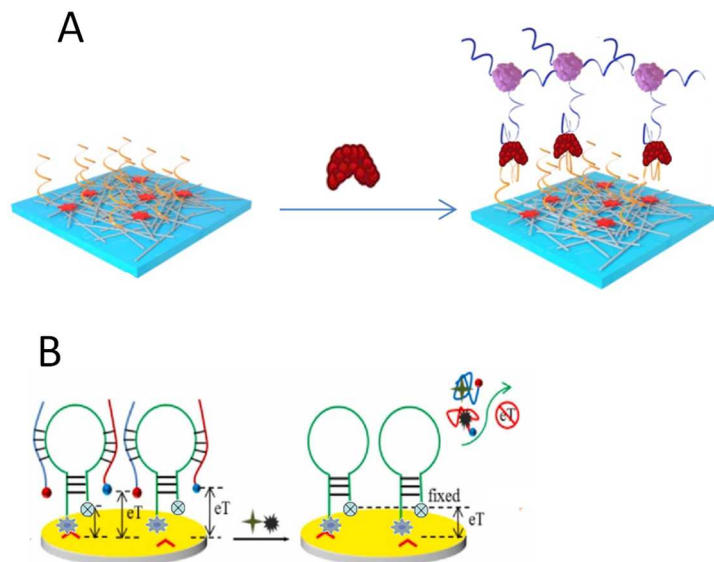
Chai *et al* [42] have developed a potassium aptasensor involving the recognition step in solution (Table 1 – Entry 4). MB was covalently attached to a thiolated guanine-rich aptamer sequence. The thiol group was hidden into a stem-loop structure preventing the anchoring of the aptamer on the gold electrode. The presence of K<sup>+</sup> induced a conformation transition of the aptamer to form a G4 structure that liberated the thiol, resulting in the assembling on the electrode surface and consequently detection of the MB label by SWV. Similar strategy was also used to detect the thrombin [45] (Table 1 –Entry 5).

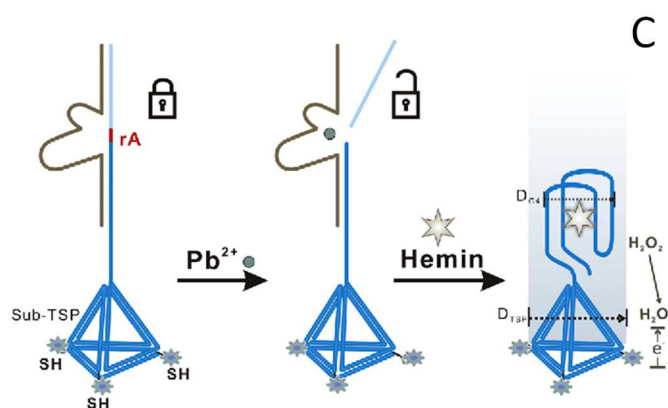




**Figure 2.** Aptasensor with a detection mode based on the conformation modification: A) without an electroactive species attached at the aptamer extremity (*i.e.* the electroactive species is in solution); B) and C) with an electroactive species attached on the aptamer. In B) the aptamer is previously immobilized before the recognition event, in C) the recognition event in solution induces the aptamer immobilization on surface. The figures A, B and C were adapted from references [35], [44] and [45] respectively. *e.t.* or *e-*: electron transfer.

For extending the variety of analytes detection, G4-based aptasensors with more complex architecture were developed. The sandwich assays are usually applied for large size analytes because it could bind more than one aptamer. In a direct sandwich assay, the analyte first binds to the sensing aptamer grafted onto the electrode surface and in a second step a secondary labeled aptamer is added (Fig. 3A). A thrombin aptasensor describing an example of sandwich structure involving two thrombin aptamers **1** and **2** has been reported (Table 1 – Entry 6). Aptamer **1** was immobilized onto an electrode previously modified with a conductive hydrogel copolymerized with polyaniline while aptamer **2** was anchored onto magnetic nanoparticles (MNP). In the presence of thrombin, aptamer **1**, thrombin and MNP-aptamer **2** could form a sandwich structure resulting in an electrochemical signal-off. In this biosensing system, both aptamers served as the biorecognition elements to specifically bind the target [9].





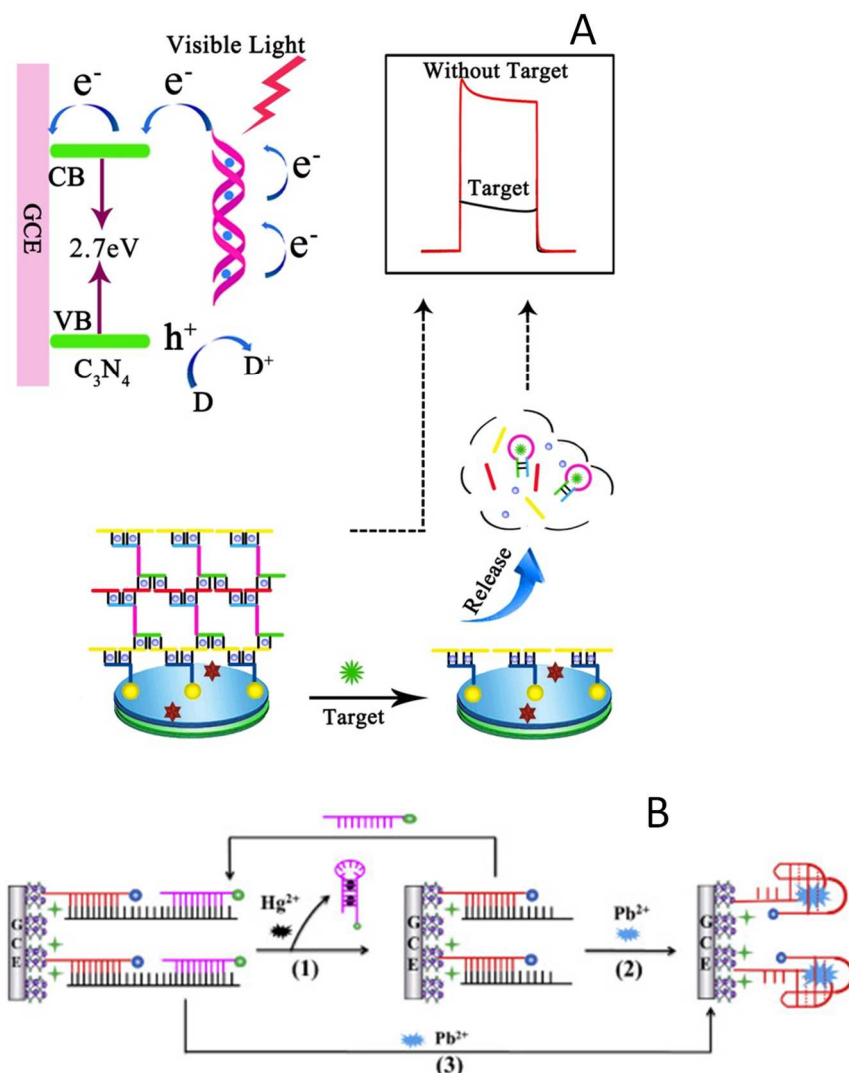
**Figure 3.** A) Example of a sandwich assay [9]; B) Design of aptasensors, with the sensing aptamer previously hybridized with a complementary strand, in which the binding of the analyte induces the denaturation of the duplex DNA [37]; C) Use of the hemin to induce the G4 formation after recognition of the target [43]. *Sub-TSP*: specific tetrahedral nanostructure-based substrate; *eT* or *e-*: electron transfer.

For some other sensing schemes using redox probes, the binding of the analyte with the sensing aptamer previously hybridized with a complementary strand, induces the denaturation of the duplex DNA and results in a change of the electrochemical signal (Fig. 3B). Zhu *et al.* [37] have developed a dual-ratiometric electrochemical aptasensing strategy based on the denaturation of a DNA duplex (Table 1 –Entry 7). The aptasensor simultaneously targeted two mycotoxins (*i.e.* ochratoxin A (OTA) and aflatoxin B1 (AFB1)) found as contaminants widespread in food products. In the designed construction, an anthraquinone (AQ)-labelled hairpin DNA was used to provide separate and specific binding sites to assemble the ferrocene-labelled AFB1 aptamer (Fc-Apt1) and methylene-blue-labelled OTA aptamer (MB-Apt2). In the presence of the analytes, the binding events induced the release of G4 Fc-Apt1 and MB-Apt2 bound to their respective analytes, leading to a decrease of the oxidation current of Fc and MB, while the oxidation of AQ remained unchanged. Thus, respective current ratios measured by ACV were used to quantify AFB1 and OTA, respectively.

Such sensing mechanism involving a competition between duplex and G-quadruplex structures fits particularly well for the case of hemin-G4 complex. The latter complex is an example of DNAzyme resulting from the interaction of hemin, an iron-containing porphyrin, with G4 structure. The hemin-G4 aptamer could act as a bioreceptor in the biosensing device for which an amplification of the electrochemical signal involves a peroxidase-like catalytic activity [47]. This property has been extensively exploited in the field of biosensors [48] and numerous example of electrochemical sensors using this peroxidase-like activity have been published [49]. For instance, it has been used for the detection of lead ( $Pb^{2+}$ ) on gold functionalized surfaces (Figure 3C) [43]. The sensor was composed of 3D-DNA tetrahedral structures to control density and orientation of the sensing nucleic acid strands (Table 1 – Entry 8). Two catalytic DNA constructs were used: the hemin/G4 complex and a lead specific DNAzyme. This latter is a duplex oligonucleotide in which one strand is able to catalyze the cleavage of the complementary substrate strand in the presence of  $Pb^{2+}$ . The substrate strand was linked to the extremity of the hemin aptamer sequence for which the formation of the G4 is precluded because of the formation of the duplex. The detection of the target occurs in two steps conformational switches: in presence of  $Pb^{2+}$ , the substrate strand is removed from the hemin aptamer allowing it to form the G4 and subsequent interaction with hemin. Then the hemin-G4 complex is able to catalyze the reduction of  $H_2O_2$  that is monitored by CV. Another example based on hemin-G4 catalyzing an electrochemical reaction is related to the detection of OTA [21]. DNA tetrahedral nanostructures (DTN) grafted on gold nanorods were modified with OTA aptamer which forms G4-hemin complex in the presence of the analyte and the interaction with hemin (Table 1 – Entry 9). The detection mechanism is based on the catalytic oxidation of aniline in the presence of  $H_2O_2$  by G4-hemin complex which amplify DPV signal. Huang *et al* [16] reported the development of a label-free aptasensor based on a hemin-G4 for the electrochemical detection of gastric cancer exosomes (Table 1 – Entry 10). The sensing platform contains an anti-CD63 antibody modified gold electrode used to capture the exosomes and an exosome specific aptamer linked to a primer sequence which is a complementary sequence to a G4 circular template. The presence of exosomes triggered rolling circle amplification and produced G4 units. The incubation with hemin induced the formation of hemin-G4 complex which is able to catalyze the reduction of  $H_2O_2$  and generates the electrochemical signal (DPV).

The co-immobilization of photoactive or luminescent nanomaterials with aptamers has paved the way to the design of new highly sensitive aptasensors. Photoelectrochemical (PEC) sensing is based on the photoinduced charge transfer from the aptamer to the electrode. It is expected to significantly increase the signal-to-noise ratio with respect to electrochemical sensing because the excitation event (by light) is decoupled from the detection event (electrochemical signal).

Da *et al.* [46] developed a sensitive signal-off PEC aptasensor based on the assembly/disassembly of an aptamer bridged 3D DNA network structure for the sensitive detection of VEGF<sub>165</sub> (Table 1 –Entry 11). By using photoactive graphitic carbon nitride electrode (GCE-C<sub>3</sub>N<sub>4</sub>), an initial photocurrent signal is generated. The DNA network provides an excellent dsDNA platform for the incorporation of MB which enhanced the PEC signal. In the presence of the VEGF<sub>165</sub>, the DNA network is destroyed through the formation of aptamer-VEGF<sub>165</sub> complex, resulting in the release of MB from the DNA network, which further induces a decrease of the PEC signal (Fig. 4A). The excellent properties endow the PEC aptasensor very broad applications in clinical diagnoses for cancers.



**Figure 4.** Schematic illustrations for the preparation and sensing mechanism of (A) a photoelectrochemical [46] and (B) a electrochemoluminescence aptasensor [25]. *CB*: conduction band; *VB*: valence band; *D*: electron donor, H<sub>2</sub>O<sub>2</sub>.

Another strategy refers to the electrochemoluminescence (ECL) for which the production of light is performed through an electrochemical reaction (Fig. 4B). Deng *et al.* [25] designed an aptasensor based on composites of metal-organic framework (MOFs) and quantum dots MIL-53(Al)@CdTe for the concomitant determination of Hg<sup>2+</sup> and Pb<sup>2+</sup> (Table 1 – Entry 12). Two different aptamers labeled with metal nanoparticles (aptamer1-AuNPs for Hg<sup>2+</sup> recognition and aptamer2-PtNPs for Pb<sup>2+</sup>) were used as bioreceptors. Upon aptamer interaction with analytes, decreased ECL intensity was observed. Indeed, the recognition of Hg<sup>2+</sup> with aptamer1-AuNPs generates a conformational switch of this one and induces its removal from the electrode, whereas the capture of Pb<sup>2+</sup> by aptamer2-PtNPs triggered its folding into G4 structure bringing the attached PtNPs close enough to CdTes quantum dots to produce a decrease of the ECL intensity owing to the ECL energy transfer between PtNPs and CdTes.

In conclusion, this short review focuses on the applications of G-quadruplex in the design of electrochemical aptasensors able to detect a wide variety of analytes including low molecular weight molecules, proteins and living cells for different applications from clinical diagnosis to environmental monitoring. The achievement of G4-based aptasensors with high sensitivity required signal amplification. Various strategies have been reported: hybridization chain reaction, rolling circle amplification, target triggered amplification, catalysis amplification or nanomaterial based

amplification. G4-based aptasensors can be used with other detection techniques such as optical methods, however electrochemical aptasensors has the advantages of simple operation and are consequently miniaturizable making them efficient candidates for on-site assay. However, the analysis time depends on the design of the aptasensors and varies from few minutes to several hours. The large incubation time could hamper their application. Several efforts are made to reduce the analysis time and enhance the limit of detection. In our opinion, the main interest of G4 aptasensors relies on the DNAzyme (hemin-G4) mediated amplification which represents a promising strategy to improve the biosensors analytical performance.

#### CRedit authorship contribution statement

J. Dejeu : Writing - Original Draft, Writing - Review & Editing; A. Van der Heyden : Writing - Review & Editing; E. Defrancq : Writing - Review & Editing; N. Spinelli : Writing - Review & Editing; L. Coche-Guérente : Writing - Original Draft, Writing - Review & Editing

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Bibliographic references:

- [1] S.M. Shaban, D.H. Kim, Recent Advances in Aptamer Sensors, *Sensors*. 21 (2021) 979. <https://doi.org/10.3390/s21030979>.
- [2] S.R. Yan, M.M. Foroughi, M. Safaei, S. Jahani, N. Ebrahimpour, F. Borhani, N.R.Z. Baravati, Z. Aramesh-Boroujeni, L.K. Foong, A review: Recent advances in ultrasensitive and highly specific recognition aptasensors with various detection strategies, *Int. J. Biol. Macromol.* 155 (2020) 184–207. <https://doi.org/10.1016/j.ijbiomac.2020.03.173>.
- [3] H. MacDonald, H. Bonnet, A. Van der Heyden, E. Defrancq, N. Spinelli, L. Coche-Guérente, J. Dejeu, Influence of Aptamer Surface Coverage on Small Target Recognition: A SPR and QCM-D Comparative Study, *J. Phys. Chem. C*. 123 (2019) 13561–13568. <https://doi.org/10.1021/acs.jpcc.9b00845>.
- [4] C. Roxo, W. Kotkowiak, A. Pasternak, G-Quadruplex-Forming Aptamers-Characteristics, Applications, and Perspectives, *Molecules*. 24 (2019) 3781. <https://doi.org/10.3390/molecules24203781>.
- [5] A. Sett, L. Zara, E. Dausse, J.-J. Toulmé, Engineering Light-Up Aptamers for the Detection of RNA Hairpins through Kissing Interaction, *Anal. Chem.* 92 (2020) 9113–9117. <https://doi.org/10.1021/acs.analchem.0c01378>.
- [6] A. Osypova, D. Thakar, J. Dejeu, H. Bonnet, A. Van der Heyden, G.V. Dubacheva, R.P. Richter, E. Defrancq, N. Spinelli, L. Coche-Guérente, P. Labbé, Sensor Based on Aptamer Folding to Detect Low-Molecular Weight Analytes, *Anal. Chem.* 87 (2015) 7566–7574. <https://doi.org/10.1021/acs.analchem.5b01736>.
- [7] J. Dejeu, H. Bonnet, N. Spinelli, E. Defrancq, L. Coche-Guérente, A. Van der Heyden, P. Labbé, Impact of Conformational Transitions on SPR Signals—Theoretical Treatment and Application in Small Analytes/Aptamer Recognition, *J. Phys. Chem. C*. 122 (2018) 21521–21530. <https://doi.org/10.1021/acs.jpcc.8b07298>.
- [8] A. Vignon, A. Flaget, M. Michelas, M. Djeghdir, E. Defrancq, L. Coche-Guerente, N. Spinelli, A. Van der Heyden, J. Dejeu, Direct Detection of Low-Molecular-Weight Compounds in 2D and 3D Aptasensors by Biolayer Interferometry, *ACS Sens.* 5 (2020) 2326–2330. <https://doi.org/10.1021/acssensors.0c00925>.
- \*[9] X.Y. Wang, F.X. Gao, Y.Y. Gong, G.T. Liu, Y. Zhang, C.F. Ding, Electrochemical aptasensor based on conductive supramolecular polymer hydrogels for thrombin detection with high selectivity, *Talanta*. 205 (2019) 120140. <https://doi.org/10.1016/j.talanta.2019.120140>.
- [10] Y.P. Song, L.N. He, K. Chen, M.H. Wang, L.Y. Yang, L.H. He, C.P. Guo, Q.J. Jia, Z.H. Zhang, Quantification of EGFR and EGFR-overexpressed cancer cells based on carbon dots@bimetallic CuCo Prussian blue analogue, *RSC Adv.* 10 (2020) 28355–28364. <https://doi.org/10.1039/d0ra01439g>.
- [11] N. Zhou, F.F. Su, C.P. Guo, L.H. He, Z.K. Jia, M.H. Wang, Q.J. Jia, Z.H. Zhang, S.Y. Lu, Two-dimensional oriented growth of Zn-MOF-on-Zr-MOF architecture: A highly sensitive and selective platform for detecting cancer markers, *Biosens. Bioelectron.* 123 (2019) 51–58. <https://doi.org/10.1016/j.bios.2018.09.079>.
- [12] Y. Wu, B. Midinov, R.J. White, Electrochemical Aptamer-Based Sensor for Real-Time Monitoring of Insulin, *ACS Sens.* 4 (2019) 498–503. <https://doi.org/10.1021/acssensors.8b01573>.
- [13] D.P. Sun, X.A. Lin, J. Lu, P. Wei, Z.B. Luo, X.E. Lu, Z.G. Chen, L.Y. Zhang, DNA nanotetrahedron-assisted electrochemical aptasensor for cardiac troponin I detection based on the co-catalysis of hybrid nanozyme, natural enzyme and artificial DNAzyme, *Biosens. Bioelectron.* 142 (2019) 111578. <https://doi.org/10.1016/j.bios.2019.111578>.



- [14] A. Diaz-Fernandez, R. Lorenzo-Gomez, R. Miranda-Castro, N. de-los-Santos-Alvarez, M.J. Lobo-Castanon, Electrochemical aptasensors for cancer diagnosis in biological fluids - A review, *Anal. Chim. Acta.* 1124 (2020) 1–19. <https://doi.org/10.1016/j.aca.2020.04.022>.
- [15] Y.M. Wu, G.P. Li, L.N. Zou, S. Lei, Q. Yu, B.X. Ye, Highly active DNAzyme-peptide hybrid structure coupled porous palladium for high-performance electrochemical aptasensing platform, *Sens. Actuator B-Chem.* 259 (2018) 372–379. <https://doi.org/10.1016/j.snb.2017.12.091>.
- \*[16] R.R. Huang, L. He, Y.Y. Xia, H.P. Xu, C. Liu, H. Xie, S. Wang, L.J. Peng, Y.F. Liu, Y. Liu, N.Y. He, Z.Y. Li, A Sensitive Aptasensor Based on a Hemin/G-Quadruplex-Assisted Signal Amplification Strategy for Electrochemical Detection of Gastric Cancer Exosomes, *Small.* 15 (2019) 1900735. <https://doi.org/10.1002/smll.201900735>.
- [17] D.P. Sun, J. Lu, D.B. Chen, Y.F. Jiang, Z.R. Wang, W.W. Qin, Y.Y. Yu, Z.G. Chen, Y.Q. Zhang, Label-free electrochemical detection of HepG2 tumor cells with a self-assembled DNA nanostructure-based aptasensor, *Sens. Actuator B-Chem.* 268 (2018) 359–367. <https://doi.org/10.1016/j.snb.2018.04.142>.
- [18] Y. Lv, S. Chen, Y. Shen, J. Ji, Q. Zhou, S. Liu, Y. Zhang, Competitive Multiple-Mechanism-Driven Electrochemiluminescent Detection of 8-Hydroxy-2'-deoxyguanosine, *J. Am. Chem. Soc.* 140 (2018) 2801–2804. <https://doi.org/10.1021/jacs.8b00515>.
- [19] B.S. He, M. Li, M.N. Li, Electrochemical determination of sulfamethazine using a gold electrode modified with multi-walled carbon nanotubes, graphene oxide nanoribbons and branched aptamers, *Microchim. Acta.* 187 (2020) 274. <https://doi.org/10.1007/s00604-020-04244-4>.
- [20] B. Deiminiati, G.H. Rounaghi, A novel visible light photoelectrochemical aptasensor for determination of bisphenol A based on surface plasmon resonance of gold nanoparticles activated g-C<sub>3</sub>N<sub>4</sub> nanosheets, *J. Electroanal. Chem.* 886 (2021) 115122. <https://doi.org/10.1016/j.jelechem.2021.115122>.
- [21] M. Wei, W.Y. Zhang, Ultrasensitive aptasensor with DNA tetrahedral nanostructure for Ochratoxin A detection based on hemin/G-quadruplex catalyzed polyaniline deposition, *Sens. Actuator B-Chem.* 276 (2018) 1–7. <https://doi.org/10.1016/j.snb.2018.08.072>.
- \*[22] Q. Yuan, S. Wu, C. Ye, X. Liu, J. Gao, N. Cui, P. Guo, G. Lai, Q. Wei, M. Yang, W. Su, H. Li, N. Jiang, L. Fu, D. Dai, C.-T. Lin, K.W.A. Chee, Sensitivity enhancement of potassium ion (K<sup>+</sup>) detection based on graphene field-effect transistors with surface plasma pretreatment, *Sensors and Actuators B: Chemical.* 285 (2019) 333–340. <https://doi.org/10.1016/j.snb.2019.01.058>.
- [23] L.Y. Wang, X.L. Peng, H.J. Fu, C. Huang, Y.P. Li, Z.M. Liu, Recent advances in the development of electrochemical aptasensors for detection of heavy metals in food, *Biosens. Bioelectron.* 147 (2020) 111777. <https://doi.org/10.1016/j.bios.2019.111777>.
- [24] A.A. Ensafi, F. Akbarian, E. Heydari-Soureshjani, B. Rezaei, A novel aptasensor based on 3D-reduced graphene oxide modified gold nanoparticles for determination of arsenite, *Biosens. Bioelectron.* 122 (2018) 25–31. <https://doi.org/10.1016/j.bios.2018.09.034>.
- \*[25] D. Feng, P. Li, X. Tan, Y. Wu, F. Wei, F. Du, C. Ai, Y. Luo, Q. Chen, H. Han, Electrochemiluminescence aptasensor for multiple determination of Hg<sup>2+</sup> and Pb<sup>2+</sup> ions by using the MIL-53(Al)@CdTe-PEI modified electrode, *Anal. Chim. Acta.* 1100 (2020) 232–239. <https://doi.org/10.1016/j.aca.2019.11.069>.
- [26] T.T. Hao, C.F. Zhang, H. Lin, W.T. Wei, F. Yang, Y.B. Wu, L.M. Niu, W.J. Kang, Z.Y. Guo, A One-Step Dual-Mode Aptasensor for Subnanomolar Detection of Lead Ions Based on Electrochemiluminescence and Fast Scan Voltammetry, *J. Electrochem. Soc.* 167 (2020) 126506. <https://doi.org/10.1149/1945-7111/abaf76>.
- [27] Y.H. Wang, F.T. Wang, Z.W. Han, K.J. Huang, X.M. Wang, Z.H. Liu, S.Y. Wang, Y.F. Lu, Construction of sandwiched self-powered biosensor based on smart nanostructure and capacitor: Toward multiple signal amplification for thrombin detection, *Sens. Actuator B-Chem.* 304 (2020). <https://doi.org/10.1016/j.snb.2019.127418>.
- [28] M. Amiri, K. Nekoueiian, R.S. Saberi, Graphene-family materials in electrochemical aptasensors, *Anal. Bioanal. Chem.* 413 (2021) 673–699. <https://doi.org/10.1007/s00216-020-02915-y>.
- [29] Y.G. Wang, G.H. Zhao, Q. Zhang, H. Wang, Y. Zhang, W. Can, N. Zhang, B. Du, Q. Wei, Electrochemical aptasensor based on gold modified graphene nanocomposite with different morphologies for ultrasensitive detection of Pb<sup>2+</sup>, *Sens. Actuator B-Chem.* 288 (2019) 325–331. <https://doi.org/10.1016/j.snb.2019.03.010>.
- [30] H. Yang, P. Hu, J. Tang, Y. Cheng, F. Wang, Z. Chen, A bifunctional electrochemical aptasensor based on AuNPs-coated ERGO nanosheets for sensitive detection of adenosine and thrombin, *Journal of Solid State Electrochemistry.* 25 (2021) 1383–1391. <https://doi.org/10.1007/s10008-021-04916-y>.
- [31] H.X. Xu, T.T. Zhang, Y. Gu, X.Y. Yan, N.N. Lu, H. Liu, Z.Q. Xu, Y. Xing, Y. Song, Z.Q. Zhang, M. Yang, An electrochemical thrombin aptasensor based on the use of graphite-like C<sub>3</sub>N<sub>4</sub> modified with silver nanoparticles, *Microchim. Acta.* 187 (2020) 163. <https://doi.org/10.1007/s00604-020-4111-4>.
- [32] S.M. Yang, Y. Teng, Q. Cao, C.P. Bai, Z.L. Fang, W.Y. Xu, Electrochemical Sensor Based on Molecularly Imprinted Polymer-Aptamer Hybrid Receptor for Voltammetric Detection of Thrombin, *J. Electrochem. Soc.* 166 (2019) B23–B28. <https://doi.org/10.1149/2.0131902jes>.
- [33] J.N. Ding, D.W. Zhang, Y. Liu, M.L. Yu, X.J. Zhan, D. Zhang, P. Zhou, An electrochemical aptasensor for detection of lead ions using a screen-printed carbon electrode modified with Au/polypyrrole composites and toluidine blue, *Anal. Methods.* 11 (2019) 4274–4279. <https://doi.org/10.1039/c9ay01256g>.
- [34] L. Wang, A.G. Wu, G. Wei, Graphene-based aptasensors: from molecule-interface interactions to sensor design and biomedical diagnostics, *Analyst.* 143 (2018) 1526–1543. <https://doi.org/10.1039/c8an00081f>.

- [35] G.J. Ran, F.M. Wu, X.Y. Ni, X.Y. Li, X.S. Li, D.L. Liu, J.X. Sun, C.F. Xie, D.S. Yao, W.B. Bai, A novel label-free electrochemical aptasensor with one-step assembly process for rapid detection of lead (II) ions, *Sens. Actuator B-Chem.* 320 (2020) 128326. <https://doi.org/10.1016/j.snb.2020.128326>.
- [36] X.Y. Liang, J.C. Zhao, Z.F. Ma, Improved binding induced self-assembled DNA to achieve ultrasensitive electrochemical proximity assay, *Sens. Actuator B-Chem.* 304 (2020) 127278. <https://doi.org/10.1016/j.snb.2019.127278>.
- \*[37] C.X. Zhu, D. Liu, Y.Y. Li, S. Ma, M. Wang, T.Y. You, Hairpin DNA assisted dual-ratiometric electrochemical aptasensor with high reliability and anti-interference ability for simultaneous detection of aflatoxin B1 and ochratoxin A, *Biosens. Bioelectron.* 174 (2021) 112654. <https://doi.org/10.1016/j.bios.2020.112654>.
- [38] P. Gupta, A. Bharti, N. Kaur, S. Singh, N. Prabhakar, An electrochemical aptasensor based on gold nanoparticles and graphene oxide doped poly(3,4-ethylenedioxythiophene) nanocomposite for detection of MUC1, *J. Electroanal. Chem.* 813 (2018) 102–108. <https://doi.org/10.1016/j.jelechem.2018.02.014>.
- [39] X.Q. Liu, Y.F. Tang, P.P. Liu, L.W. Yang, L.L. Li, Q.Y. Zhang, Y.M. Zhou, M.Z.H. Khan, A highly sensitive electrochemical aptasensor for detection of microcystin-LR based on a dual signal amplification strategy, *Analyst.* 144 (2019) 1671–1678. <https://doi.org/10.1039/c8an01971a>.
- [40] Q.X. Zhang, W.K. Li, F.Y. Zhao, C. Xu, G.C. Fan, Q.Y. Liu, X.X. Zhang, X. Zhang, Electrochemical sandwich-type thrombin aptasensor based on silver nanowires & particles decorated electrode and the signal amplifier of Pt loaded hollow zinc ferrite, *Colloid Surf. A-Physicochem. Eng. Asp.* 611 (2021) 125804. <https://doi.org/10.1016/j.colsurfa.2020.125804>.
- [41] Y. Feng, S. Xiao, X.L. Xiong, H.L. Wang, F.K. Kong, Y. Li, Y. Zhang, L.C. Chen, An impedimetric Aptasensor based on a Novel Line-Pad-Line Electrode for the Determination of VEGF(165), *Electroanalysis.* 32 (2020) 1843–1849. <https://doi.org/10.1002/elan.202060030>.
- \*[42] H. Chai, X.Y. Ma, F.Y. Meng, Q. Mei, Y.G. Tang, P. Miao, Electrochemical aptasensor based on a potassium ion-triggered DNA conformation transition and self-assembly on an electrode, *New J. Chem.* 43 (2019) 7928–7931. <https://doi.org/10.1039/c9nj00158a>.
- \*[43] L. Wang, Y. Wen, L. Li, X. Yang, N. Jia, W. Li, J. Meng, M. Duan, X. Sun, G. Liu, Sensitive and label-free electrochemical lead ion biosensor based on a DNAzyme triggered G-quadruplex/hemin conformation, *Biosensors and Bioelectronics.* 115 (2018) 91–96. <https://doi.org/10.1016/j.bios.2018.04.054>.
- [44] H. Abu-Ali, A. Nabok, T.J. Smith, Development of Novel and Highly Specific ssDNA-Aptamer-Based Electrochemical Biosensor for Rapid Detection of Mercury (II) and Lead (II) Ions in Water, *Chemosensors.* 7 (2019) 27. <https://doi.org/10.3390/chemosensors7020027>.
- [45] Y. Fan, F.S. Liao, W.T. Wu, N. Hong, L. Cheng, Y.Q. Zhong, H.F. Cui, G.Q. Ma, G.B. Wei, J. Xiong, H. Fan, A novel modification-free thrombin electrochemical sensing based homogeneous strategy, *Sens. Actuator B-Chem.* 318 (2020) 128045. <https://doi.org/10.1016/j.snb.2020.128045>.
- [46] H. Da, H. Liu, Y. Zheng, R. Yuan, Y. Chai, A highly sensitive VEGF165 photoelectrochemical biosensor fabricated by assembly of aptamer bridged DNA networks, *Biosens. Bioelectron.* 101 (2018) 213–218. <https://doi.org/10.1016/j.bios.2017.10.032>.
- [47] P. Travascio, Y. Li, D. Sen, DNA-enhanced peroxidase activity of a DNA aptamer-hemin complex, *Chem. Biol.* 5 (1998) 505–517. [http://dx.doi.org/10.1016/S1074-5521\(98\)90006-0](http://dx.doi.org/10.1016/S1074-5521(98)90006-0).
- [48] J. Kosman, B. Juskowiak, Peroxidase-mimicking DNAzymes for biosensing applications: A review, *Anal. Chim. Acta.* 707 (2011) 7–17. <http://dx.doi.org/10.1016/j.aca.2011.08.050>.
- [49] G. Pelossof, R. Tel-Vered, J. Elbaz, I. Willner, Amplified Biosensing Using the Horseradish Peroxidase-Mimicking DNAzyme as an Electrocatalyst, *Anal. Chem.* 82 (2010) 4396–4402. <https://doi.org/10.1021/ac100095u>.

