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Impedimetric Aptamer-Based Biosensors: Applications

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Contents

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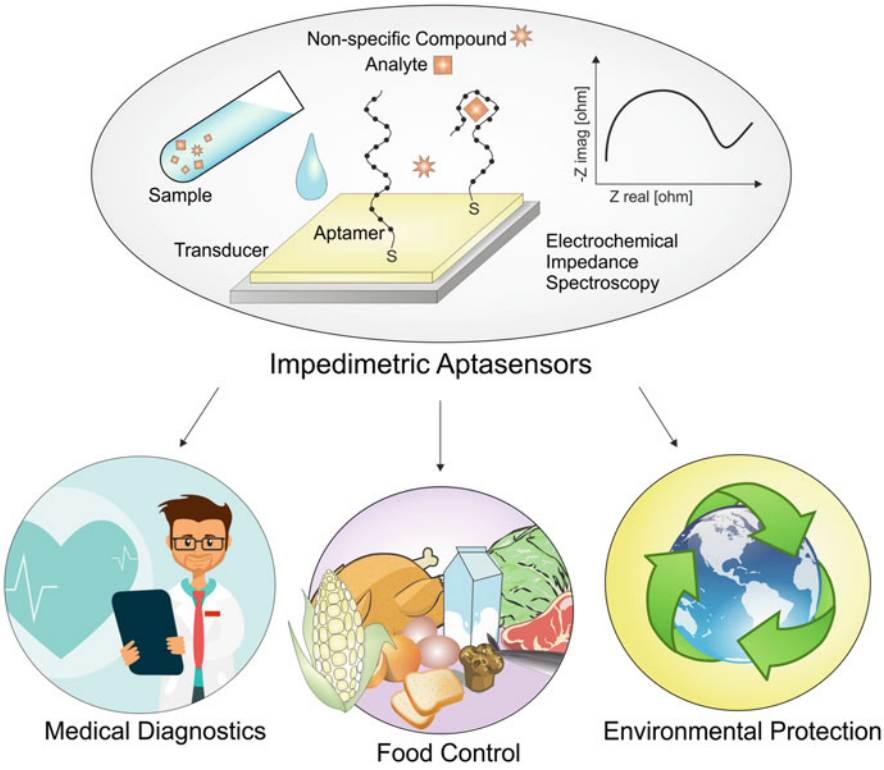
Abstract Impedimetric aptamer-based biosensors show high potential for handheld devices and point-of-care tests. In this review, we report on recent advances in aptamer-based impedimetric biosensors for applications in biotechnology. We detail on analytes relevant in medical and environmental biotechnology as well as food control, for which aptamer-based impedimetric biosensors were developed. The reviewed biosensors are examined for their performance, including sensitivity, selectivity, response time, and real sample validation. Additionally, the benefits and challenges of impedimetric aptasensors are summarized.

Graphical Abstract



Keywords Aptamer, Aptasensor, Bacteria, Biomarker, Biosensor, Cancer, Contaminants, Diagnostics, Disease, Drug, Environmental, Food industry, Health care, Impedance, Infection, Monitoring, Pathogen, Point-of-care, Serum, Therapy, Toxins, Tumor marker, Virus

1 Introduction

The main focus of this book chapter is on the review of impedimetric aptasensors since 2005 for their possible applications in biotechnology, in particular in the fields of health care, food inspection, and environmental protection (Sects. 2–4) with emphasis on target type, limit of detection, and real sample testing. Their performance will be compared to other biosensors to discuss, which benefits they offer and which challenges still have to be taken for their introduction into market (Sect. 5). Although several academic databases and search engines were sifted, some articles might have been overlooked.

2 Impedimetric Aptasensors for Health Care Applications (Red Biotechnology)

Red biotechnology aims to advance the application of biotechnological tools to benefit (bio)medical purposes. Besides developing and providing drugs, a major purpose of red biotechnology addresses the field of medical diagnostics. Instrumental diagnostic procedures are necessary in order to derive reliable diagnoses and thereby specific therapy decisions. With regard to red biotechnology, the analysis of usually complex samples (e.g. whole blood) often requires multiple processing steps in standard laboratories which demand time, highly trained personnel, and money. An ideal sensor offers cost-efficient, selective, and rapid measurements of non-processed as well as low-volume samples. Point-of-care tests point toward such specifications and enable rapid detection of analytes on-site, which can be crucial depending on the specific disease as well as the medical supply available to the patient. Readouts of point-of-care tests by smartphone applications might be the highlight of the development of simplified analysis and exchange of information with even distant professionals. The crucial question is if impedimetric aptasensors match these requirements.

Impedimetric aptasensors combine the advantages of aptamers with the advantages of EIS (electrochemical impedance spectroscopy). EIS-based sensors are promising with regard to simplified diagnostics due to rapid and label-free application without complex sample preparation. Based on the sensitivity of impedance changes at the interface of electrode and electrolyte, even the detection of analytes in the pM range is feasible. The selectivity of the sensor is ensured by aptamers which are short, stable and cost-efficient molecules [1–3].

This paragraph reviews recent publications in the field of aptamer-based impedimetric biosensors targeting medically relevant analytes, grouped by their application in the diagnosis of diseases, cancer detection, and other use cases. This review shows the potential of impedimetric aptasensors for detection and analysis of a variety of targets (e.g. proteins, cells, small molecules) from even complex origin (e.g. serum, plasma, urine).

2.1 Diagnosis of Diseases

The body's health status can be portrayed by the quantitative determination of different biomarkers which include hormones or infection-related components. Depending on the application, the diagnostic tool can facilitate a rapid alert system by the determination of general biomarkers such as the inflammatory marker CRP (C-reactive protein) or specific biomarkers for a virus type during the early stage of disease progression (see Fig. 1). Thereby, such sensitive and rapid diagnostic tools may support initial clinical examination as well as long-term monitoring of a condition, with regard to progression, recession, or recurrence.

C-reactive protein (CRP) is an unspecific biomarker that detects the risk for cardiac disease or inflammation at an early stage. Thereby, concentrations below 8 nM CRP in blood point to a low risk while concentrations above 25 nM CRP indicate a high risk [5].

So far, only non-faradaic impedimetric aptasensors have been developed for the detection of CRP but have not yet been applied in a clinically relevant matrix (serum, blood, plasma, urine, saliva). An overview of affinity-based detection methods is presented in Table 1. Qureshi et al. presented an RNA-aptamer-based sensor with gold-interdigitated capacitor arrays for the purpose of surface maximization [6]. A linear range of 4–20 pM was achieved. In the process of sensor optimization, the authors showed the influence of AC frequency on the aptamer-CRP-complex

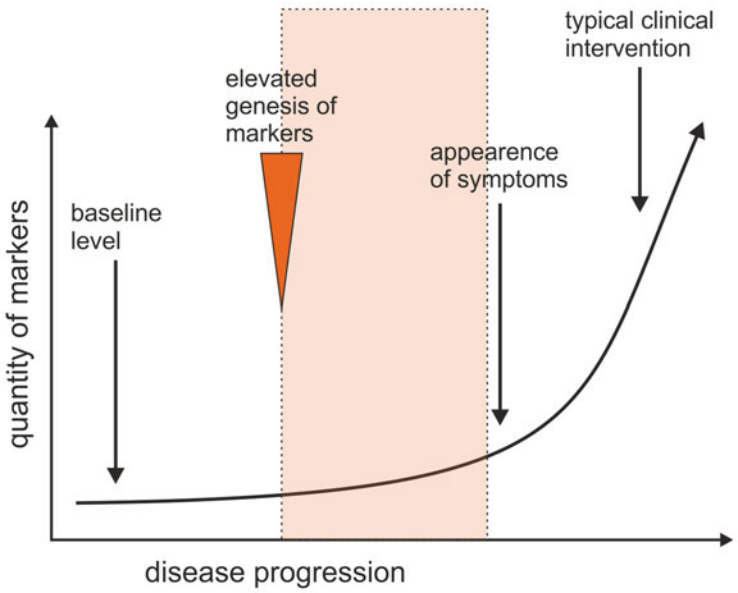


Fig. 1 The progression of a disease. A rapid increase of severity after appearance of symptoms requires more sensitive diagnostic tools facilitating detection on the very beginning of biomarker genesis. (Adapted from [4]; copyright John Wiley & Sons, Inc.)

Table 1 Biosensors for CRP determination using different methods

Method	Biorecognition element	Sample	LoD	Linear range	Reference
EIS	Aptamer	Buffer	4 pM	4–20 pM	[6]
EIS	Aptamer	Buffer	1 pM	10^1–10^4 pM	[7]
SPR	Antibody	Serum	0.36 nM	0.24–2,800 nM	[8]
EIS	Antibody	Serum	176 pM	0.5–50 nM	[9]
EIS	Antibody	Serum	176 pM	0.5–70 nM	[10]
SPR	Aptamer-antibody-Sandwich	Diluted serum	10 pM	10 pM to 100 nM	[11]
LMR	Aptamer	Buffer	2.5 nM	2.5–40 nM	[12]

SPR surface plasmon resonance, *EIS* electrochemical impedance spectroscopy, *LMR* lossy mode resonance

formation. Depending on the AC frequency, the dissociation constant changed in a non-linear manner. Hence, the impact of an electric field on binding events of aptamer and target should be considered during the experiments. In 2018, Piccoli et al. presented another aptasensor based on capacity measurements with a linear range of 10–10^4 pM and no interference with HSA [7].

Lysozyme, also called the body's own antibiotic, plays a key role in the innate human immune system [13]. It protects the organism from infections by gram-positive bacteria by hydrolyzing the murein in the cell walls. Muramidase lysozymes are especially present in secretions such as tears or saliva.

Lysozymes can serve as medical biomarkers with regard to several infections such as AIDS (acquired immune deficiency syndrome), malaria, and bacterial meningitis as well as autoimmune Alzheimer's disease or rheumatoid arthritis [13]. Lysozyme concentration in the saliva and serum of healthy patients ranges from 32 to 207 nM, while an increase points to different disorders. Herein, the potential of impedimetric aptasensors for lysozyme quantification will be discussed and compared to other biosensors (see Table 2).

Similar strategies have been published by Peng et al. and Xia et al. enabling lysozyme detection [14, 15]. The aptamer is hybridized on an immobilized, complementary strand. By addition of 100 µl sample containing lysozyme, the aptamer is released (target induced dissociation). The regeneration requires rehybridization of an aptamer sequence after formamide treatment. Mentionable features of the

Table 2 Biosensors for lysozyme determination using different methods

Method	Biorecognition element	Sample	LoD	Linear range	Reference
EIS	Aptamer	Buffer	0.2 nM	0.2–100 nM	[14]
EIS	Aptamer	Buffer	0.07 nM	0.2–4 nM	[15]
EIS	Aptamer	Buffer	1.04 pM	3.5–70 pM	[16]
EIS	Aptamer	Buffer	10 fM	0.1–500 pM	[17]
EIS	Aptamer	Buffer	6.3 nM	6.3–70 nM	[18]
ECL	Aptamer	Spiked serum	0.4 fM	3.5 fM to 350 pM	[19]

EIS electrochemical impedance spectroscopy, *ECL* electrogenerated chemiluminescence

approach by Peng et al. are a bulge of the cDNA during hybridization which increases the charge-transfer resistance as well as a covalently attached ferrocene. Their design results in a linear range of 0.2–100 nM in a buffer system, while Xia et al. enabled a linear range of 0.2–4 nM and a limit of detection (LoD) of 70 pM [14, 15]. Additionally, Xia et al. have shown the impact of incubation time. Maximal charge-transfer resistance was achieved after 80 min of incubation [15].

In order to achieve improved fabrication of the functionalized electrode with regard to higher reproducibility and automation, Khan et al. deposited carbon-nanotube-aptamer complex by inkjet printing (see Fig. 2). The anionic character of DNA results in a high charge-transfer resistance and a binding event decreases this resistance. A detection limit of 6.3 nM could be measured [18]. Hence, the approach is a seldom example of lowered impedance due to a binding event. By immobilizing the aptamer on gold nanoparticles deposited on a gold electrode, a linear range of 0.1–500 pM and a LoD of 10 fM has been achieved [17].

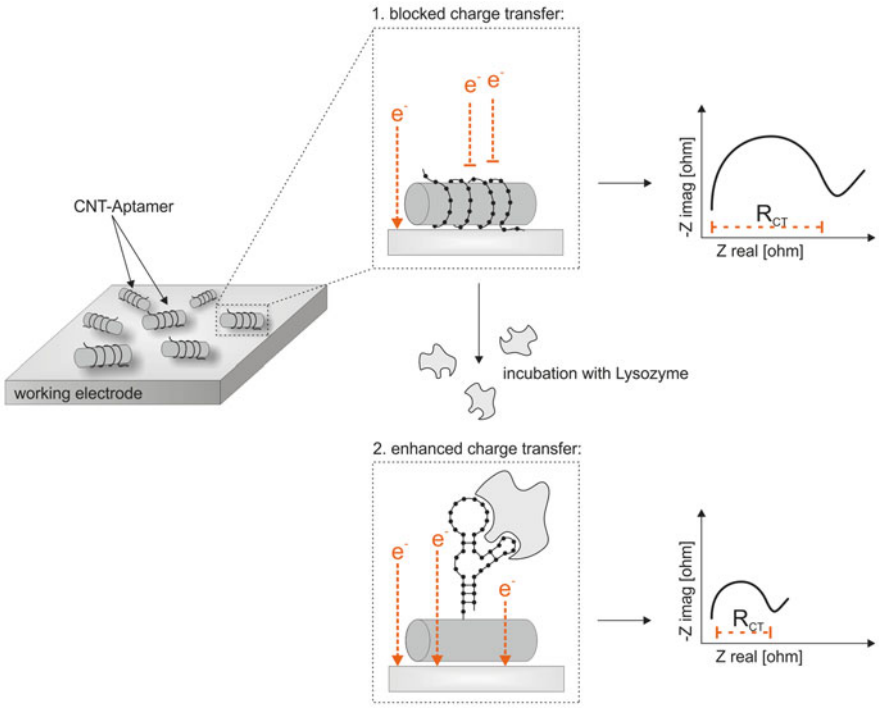


Fig. 2 Instead of a direct immobilization of the aptamer, a carbon-nanotube-aptamer-complex (CNT-Aptamer) is inkjet-printed on the working electrode. The complex is based on the high affinity between single-stranded DNA and carbon nanotubes. The anionic character of DNA results in a high charge-transfer resistance and binding of the positively charged analyte decreases this resistance. (Adapted from [18] under the CC BY license (http://creativecommons.org/licenses/by/4.0/), licensed under CC BY by SciGraphics)

In conclusion, several groups have reported aptamer-based impedimetric biosensors for lysozyme detection at picomolar concentrations (see Table 1). By using electrochemical chemiluminescence and aptamers as recognition element Dong et al. showed an astonishing detection limit of 0.4 fM in spiked serum [19]. Due to the high sensitivity of EIS aptasensors presented by Chen et al. and Zhang et al. which is magnitudes lower than the physiological concentrations, sample dilution in an appropriate buffer might be a promising strategy. Nonetheless, a proof of concept in a diluted clinical sample still needs to be provided.

Thrombin, as coagulation agent in human blood, plays a central role in human physiology. Usually, thrombin levels are low and additionally inhibited by anti-thrombin agents in order to prevent non-functional blood clogging (thrombosis). Moreover, thrombin is involved in different neurodegenerative diseases such as Alzheimer's disease or Parkinson [20].

To increase sensitivity, signal amplification is a possible strategy. For instance, a strategy presented by Deng et al. utilized the two binding sites of thrombin and consists of a multiple step detection enhancement [21]. First, thrombin is captured by immobilized aptamers on a gold surface. Second, gold nanoparticles coupled with aptamers build a sandwich with captured thrombin. Third, the gold nanoparticles serve as seed for further nanoparticle growth and thus increased steric hindrance (reduction of $HAuCl_4$). Fourth, SDS (sodium dodecyl sulfate) builds a self-assembled monolayer on the gold nanoparticles. Their negative charge further enhances the charge-transfer resistance resulting in a linear range from 50 pM up to 35 nM (LoD 100 fM) and showing fair recoveries in spiked serum.

Lu et al. developed a design suitable for EIS as well as ECL determination with the same aptamer sequence as Deng et al. The aptamer is immobilized on a gold electrode while a complementary strand coupled to a Quantum Dot is supposed to be released in the presence of thrombin (target induced dissociation). Thus, the impedance increases while the ECL signal decreases. The limit of detection of both measurement strategies is 2.7 aM. Both measurement methods result in wide linear ranges from 2.7 aM up to 2.7 μM for EIS and 2.7 aM up to 27 nM for ECL [22]. Although there is no investigation in serum, it might work since the same sequence was applied by Deng et al. for biosensor experiments in human serum.

An aptasensor based on another sequence was reported by Heydari-Bafrooei et al. in 2016 [23]. Based on a nanocomposite consisting of TiO_2, MWCNT (multiwalled carbon nanotube), chitosan, and a Schiff base applied on a glassy carbon electrode, a LoD of 1 fM was achieved due to increased surface (linear range from 50 fM up to 10 nM). The aptamer is immobilized by simple π-π stacking. Compared to an ELISA kit on healthy serum and serum of patients with different diseases (e.g. Parkinson, Epilepsy), similar results were obtained showing the applicability of the design even in complex matrices.

Interleukins, member of the cytokines, are peptide hormones that regulate immune cells. The analysis of different interleukin levels in human blood allows inferences of cell state and cell-to-cell communications.

For instance, interleukin 17A (IL17A) is a biomarker for different autoimmune diseases like arthritis or multiple sclerosis [1]. The synthesis of IL17A strictly

correlates to the synthesis of its corresponding receptor interleukin 17RA [24] and therefore is a promising target. Jo et al. presented a simple impedimetric aptasensor which is based on the coupling of thiol-modified aptamers on the gold electrode with deposited gold nanoparticles that are supposed to increase the surface for enhanced sensitivity. The approach enabled selective target recognition with regard to other interleukin proteins and a linear range of approx. 10–40 fM [1].

The pro-inflammatory Interleukin 6 is thought to play a role in the occurrence of major depression as it influences the neurotransmitter metabolism and neural plasticity [25]. Tertiş et al. applied a screen-printed carbon electrode with a film of polypyrrole and gold nanoparticles. A detection limit of 14 fM and a linear range from 42 fM to 633 nM cover the essential range [25].

Troponin I gained due to its high specificity for heart damages high relevance as a biomarker for early detection of, e.g., acute myocardial infarction. Antibody-based tests, usually ELISA and radioimmunoassay, generally lack thermal stability, require a complex sample preparation, and are cost-inefficient, albeit being sufficiently specific and sensitive [26]. Troponin I concentrations below 24.9 pM represent normal level while high concentrations above 70 pM are indicative for cardiac tissue damage [27].

In 2015, Jo et al. presented an electrochemical aptasensor that is able to quantify Troponin I with a linear range of 2 nM to 2 μM in human serum [26]. Wang et al. introduced a 13-mer peptide aptamer, immobilizing Troponin I on deposited gold nanoparticles by using the thiol-functionality of the terminal cysteine [28]. This approach achieved a linear range of 0.7–700 pM. Troponin I detection from clinical samples showed fair recoveries of 91–105%.

Higher sensitivity was achieved by Akter et al. in 2017 by an antibody-based EIS sensor [29]. Dendrimers between the electrode and the aptamer enhanced the performance resulting in even lower detection limits of 11.7 fM and a linear range of 46 fM to 46 nM. The lowest concentration measured in spiked serum was 460 fM.

Malaria results from an infection caused by parasites belonging to the *Plasmodium* family. *P. falciparum* and *P. vivax* are the main species which are transferred by the *Anopheles* mosquito. In 2016, 216 million individuals were affected by malaria and 445,000 died from it (see WHO malaria report 2017). Recommended by the WHO as state-of-the-art blood diagnostics are microscopy and rapid diagnostic testing (RDTs) [30]. However, both methods show significant disadvantages: While microscopy requires time and well-trained experts, RDTs based on antibodies against *Plasmodium* lactate dehydrogenase (LDH) show limitations with regard to costs, sensitivity at low concentrations, specificity, and thermal stability [31]. Nonetheless, since *Plasmodium* LDH is expressed in the sexual as well as the asexual stage of the parasite, it is a reliable target for affinity-based sensors.

Lee et al. and Figueroa-Miranda et al. presented impedimetric aptasensors for *Plasmodium* LDH determination from patient's blood and diluted human serum [31, 32]. In this way, they were able to distinguish between infected and uninfected blood. A simple design based on co-immobilization of 6-mercapto-1-hexanol and thiolated aptamer on a gold electrode resulted in a linear detection range of 1–1,000 pM [31]. Figueroa-Miranda et al. reported a linear range of 1 pM to 10 nM of the

Plasmodium LDH covering the necessary range from 13 to 206 pM. Depending on the pH, the required linear range can be adjusted. Moreover, the regeneration of the sensor was demonstrated [32].

For *influenza* virus *H1N1*, Kiilerich-Pedersen et al. presented an impedimetric aptasensor that is potentially applicable as a point-of-care test [33]. Standard methods include RT-PCR, antigen staining, and virus cultivation. Especially virus cultivation requires several days and is therefore not suitable for rapid testing. However, by way of example, an influenza virus that is responsible for a potentially pandemic flu, as arisen in 2009, needs to be determined in a rapid and reliable manner. In a buffer system, a linear range of 10^1–10^6 Plaque-forming units (PFU) per ml was achieved. Briefly, a suitable DNA-aptamer was immobilized on a conductive polymer in a microchannel. A saliva sample spiked with 10^3 PFU/ml (100-fold lower than in real samples) caused a signal within 15 min, which was not induced by a H1N1 virus-free saliva sample.

Cyclic adenosine monophosphate (cAMP) is a secondary messenger with various functions in different physiological contexts [34]. cAMP levels are usually low and stable, while an increase in urine or plasma points to different diseases. The detection of cAMP is an example of detecting relatively small molecules by means of impedance increase. Zhao and coworkers applied a RNA aptamer in a simple approach that uses gold electrodes [35]. These gold electrodes were enhanced by gold nanoparticles that allowed for the detection of cAMP in buffer and spiked diluted serum. In spiked serum, a linear range of 50 nM to 1 μM was achieved. Three orders of magnitude lower, in a linear range of 50–250 pM, were detectable in a buffer system. Selectivity was proven, as the sensor was not triggered by ATP, AMP, or c-diGMP.

For the detection of *adenosine*, Wang et al. used a methodically similar design [36]. The study demonstrates the influence of backfiller such as DTT and MCH which are added to functionalized aptamer solutions (see Fig. 3). Depending on the backfiller composition, linear ranges of 0.1–61 nM for pure MCH backfilling (LoD 0.03 nM), 0.5–27 pM for pure DTT backfilling (LoD 0.2 pM), and 0.05–17 pM for a mixture of DTT and MCH (LoD 0.02 pM) were achieved. For testing clinical suitability, diluted clinical samples were analyzed, resulting in recoveries of 95–101% (lowest sample concentration 0.066 pM).

2.2 Cancer Detection

The uncontrolled proliferation of the body's owns cells and the inhibition of natural cell death (apoptosis) as present in cancer require early diagnosis in order to prevent the formation of metastases. Since aptamer sequences with a high and specific affinity to different tumor-related markers and cancer cells have been selected lately, the development of impedimetric aptasensors for the detection of low level tumor markers at an early point is pursued [37, 38]. Beside the benefits of specific

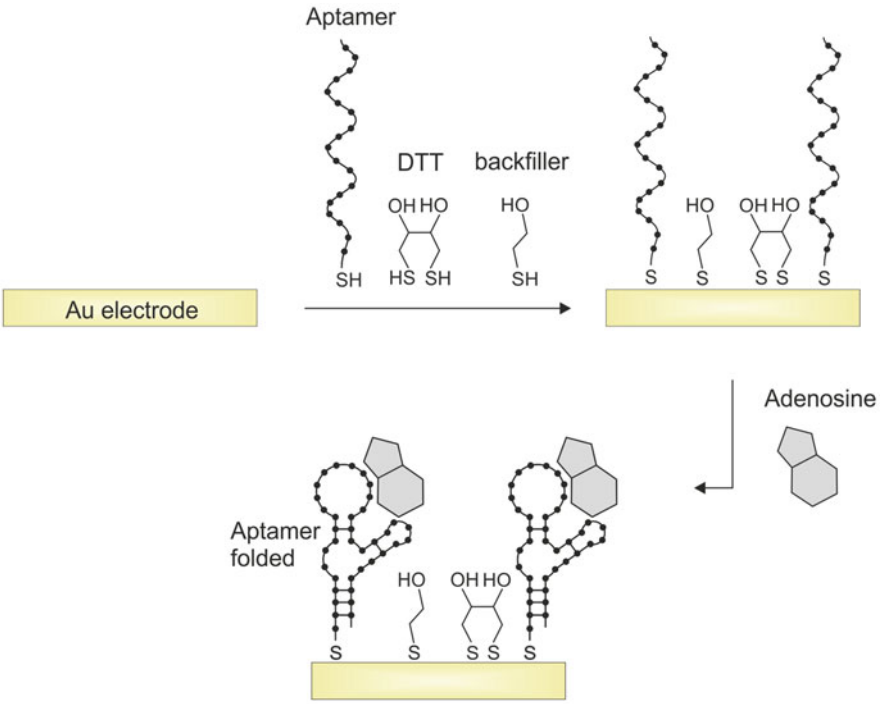


Fig. 3 Gold electrodes are often functionalized by well-known gold sulfur chemistry. In order to achieve a self-assembled monolayer (SAM) and to reduce non-specific binding, the application of short backfillers is a common strategy besides co-immobilization or post-functionalization of SAM. The impact of backfiller composition should be taken into account. (Adapted from [36], with permission from Elsevier)

diagnostics, this chapter deals with the potential of impedimetric aptasensors for aptamer-based cancer treatment by oncolytic virus.

2.2.1 Cancer Cells

The state-of-the-art diagnostics of cancer types that are based on imaging methods are limited since they mainly analyze morphology. However, morphology-based classification does not allow for early-stage-diagnosis as morphologic changes do not occur right away. As an alternative, impedimetric aptasensors are an opportunity of rapid and label-free detection of cancer cells themselves. For instance, CT26 (colorectal cancer) were detected by an impedimetric aptasensor as a rapid and label-free approach [39]. The proposed sensor enabled LoD of 2 cells/ml and a linear range of 1×10^5–6×10^6 cells/ml.

Diagnosis of hepatocellular carcinoma (HCC) goes along with poor prognosis for survival the next 5 years due to the lack of effective treatment strategies. So far, the

majority of patients are diagnosed at a late stage, because α-fetoprotein, considered to be the most useful HCC marker in human serum, lacks sensitivity and specificity as increased concentrations might be due to other liver related diseases [41]. A more specific and reliable tool for diagnostics might be the direct detection of cancer cells as published by Kashefi-Kheyrabdi et al., who proposed an impedimetric aptasensor for quantification of HepG2 cells which are a hepatocellular carcinoma cell line [38]. A LoD of 2 cells/ml and a linear range of 10^2–10^6 cells/ml were achieved based on the covalent immobilization of aptamer molecules on a gold electrode.

Since the blood of tumor patients contains low levels of circulating tumor cells (CTCs), screening of isolated CTCs enables diagnosis at early stage. Shen et al. [40] presented an approach that focuses on the reuse of the biosensor surface and the collection of viable cells after detection [40]. The authors used an aptamer that targets an epithelial adhesion molecule that is specifically overexpressed in breast cancer cells. The sensor design consisted of an immobilized capture probe complementary to the aptamer. The hybridized aptamers captured the CTCs and the impedance is measured. A uracil excision enzyme specifically digested the aptamer, leaving the DNA capture probe intact for further experiments and the isolated cells are released for further cultivation (see Fig. 4). With regard to the CTC, a LoD of 10 cells/ml and a high linear range of 30–10^6 cells/ml were achieved.

2.2.2 Soluble Tumor Marker

The *carcinoembryonic antigen (CEA)* is an example of a protein tumor marker that is quantifiable by impedimetric aptasensors. Despite its unspecificity, CEA plays an important role in diagnostics and monitoring. CEA is expressed on the tumor membrane and additionally secreted into the blood. Normal concentrations of CEA in the blood range from 3 to 5 ng/ml; increased CEA levels indicate tumor progression or recurrence [42, 43].

Different biosensors for CEA detection have recently been published as summarized in Table 3. Shekari et al. developed a selective aptasensor with a linear range of 1 pg/ml to 100 ng/ml [43]. An even lower linear range of 0.1 fg/ml to 1 pg/ml was achieved by Wang et al. in 2015 by excluding the usual need of covalent immobilization [44]. The required sample volume was 50 µl. The working principle engages the differences of adsorption from ssDNA and dsDNA on graphene surfaces when target binding to the single-stranded aptamer induces dsDNA formation. The lowered charge-transfer resistance results from dsDNA desorption. Since the linear range falls far below the diagnostic range, the required dilution of serum is not limiting. In 2017, Guo et al. reported about a design based on silver nanoclusters and aptamers embedded in zirconium metal-organic clusters [45]. Achieving a linear range of 10 pg/ml up to 10 ng/ml (LoD: 5 pg/ml) in spiked serum, the approach potentially enables appropriate diagnostics. Good repeatabilities ($n = 5$), selectivity, lifetime (12 days), and possible regeneration ($n = 8$) were achieved. Just the sample incubation time of 3 h might be limiting with regard to rapid diagnostics.

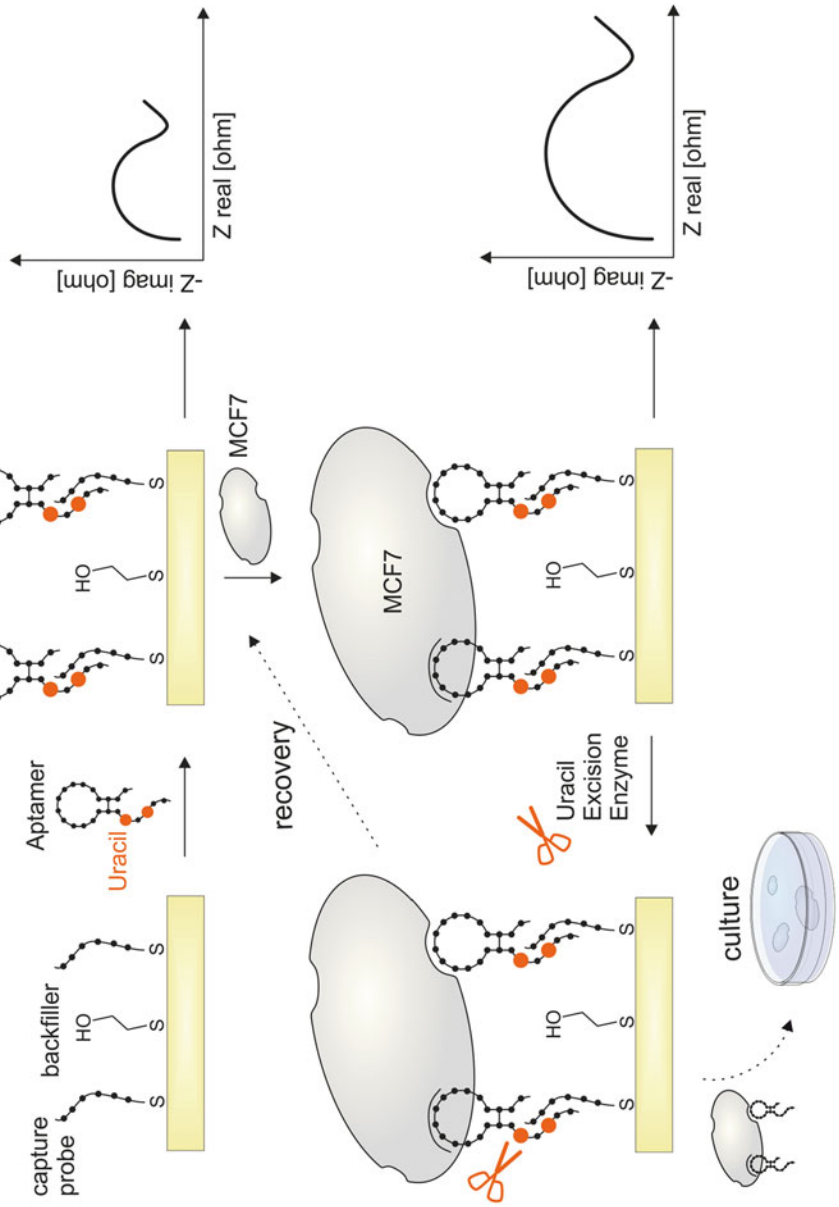


Fig. 4 Design for detection and capture of CTCs by an impedimetric aptasensor: Aptamers are hybridized to complementary DNA capture probes on the sensor surface. Then, CTCs from the sample bind to these aptamers and an increase in impedance is measured. Cells are released by the application of a uracil excision enzyme, specifically targeting the aptamer due to its uracil bases. Recovered CTCs can be used for further experiments and the recovered sensor can be reused. (Adapted from [40], with permission from Elsevier)

Table 3 Different biosensors for CEA detection

Method	Biorecognition element	Sample	LoD	Linear range in g/ml	Reference
EIS	Aptamer	Diluted serum	1 pg/ml	1 p to 100 n	[43]
EIS	Aptamer	Diluted serum	0.1 fg/ml	0.1 f to 1 p	[44]
EIS	Aptamer	Spiked serum	5 pg/ml	0.01–10 n	[45]
DPV	Aptamer	Human serum	3.4 ng/ml	5–40 n	[46]
ELISA	Antibody	Spiked plasma	2 ng/ml	2–64 n	[47]

DPV differential pulse voltammetry, *ELISA* enzyme-linked immunosorbent assay, *EIS* electrochemical impedance spectroscopy

The *vascular endothelial growth factor (VEGF)* is an important biomarker for cancer diagnostics as well as for other diseases [48, 49]. Secreted into the blood for the purpose of angiogenesis, healthy individuals have serum concentrations of 1–177 pg/ml VEGF while individuals with cancer show a serum level of 18–328 pg/ml VEGF due to increased tissue formation [50]. Therefore, highly sensitive detection methods are required in order to detect the fine differences between healthy and cancerous VEGF blood concentrations [51].

In 2015, two studies were published presenting impedimetric aptasensors for VEGF detection in human serum. Tabrizi et al. reached a linear range of 10–300 pg/ml and a detection limit of 1 pg/ml by using mesoporous gold nanocomposites on a screen-printed electrode [49]. For the immobilization, the affinity of gold toward thiolated aptamer molecules was used. As proof for a potential application, diluted serum of a patient with lung cancer was tested. The value measured with a standard ELISA at the local hospital could be recovered to 97% by the developed biosensor.

A more exotic approach was proposed by Qureshi et al. who designed a non-faradaic aptasensor. Instead of impedance change, this sensor used changes in capacitance [48]. A linear detection range of 400 pg/ml up to 1 ng/ml VEGF in spiked serum was obtained for a low volume sample of 5 µl (LoD: 5 pg/ml). The authors showed the increased capacitance change by a sandwich assay design compared to a simple aptamer immobilized on a gold surface. The sandwich assay additionally included an antibody linked to a magnetic bead (see Fig. 5). The approach is promising with regard to potential parallelization and the need of low volumes. However, the complexity of the approach might be limiting due to the need of an additional element such as an eventually expensive antibody. Moreover, the antibody must not bind the same epitope as the aptamer. The approaches presented by Tabrizi et al. and Qureshi et al. are close to the medically relevant range.

Compared to other VEGF-biosensors, the presented impedimetric biosensors stand out due to its high sensitivity even in a complex biological matrix (see Table 4). Da et al. presented an aptasensor based on photoelectrochemical detection

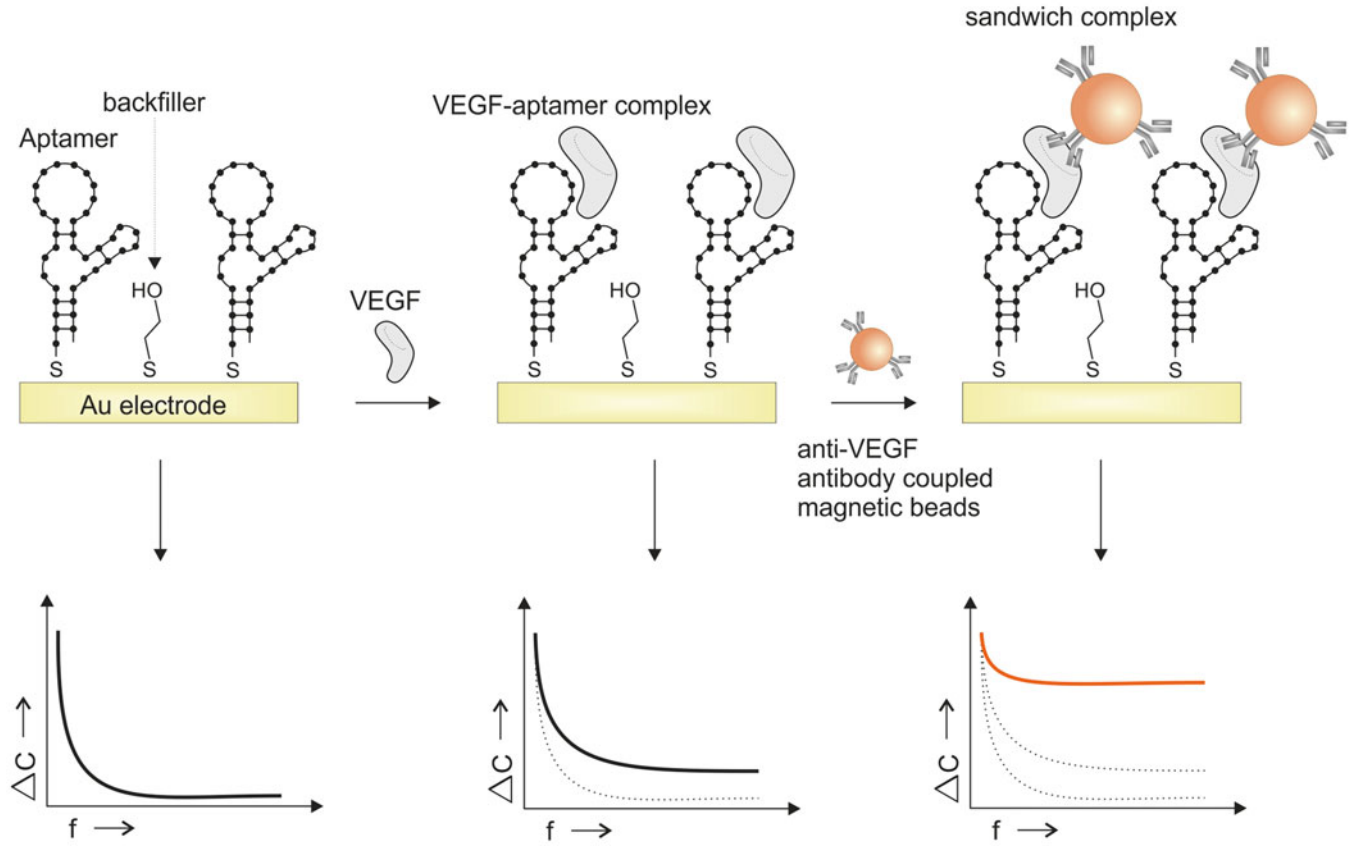


Fig. 5 An aptamer immobilized on a gold electrode binds VEGF. An anti-VEGF antibody coupled with magnetic beads used to build a sandwich and to further increase the capacitance. (Adapted from [48], with permission from Elsevier)

Table 4 Different biosensors for VEGF determination

Method	Biorecognition element	Sample	LoD in pg/ml	Linear range in g/ml	Reference
EIS	Aptamer	Diluted serum	1	10–300 p	[49]
EIS	Aptamer-antibody	Spiked serum	5	400 p to 1 n	[48]
EIS	VEGF receptor 1	Artificial serum	100	100–700 p	[52]
DPV	Antibody	Human serum	50	50 p to 100 n	[53]
PEC[a]	Aptamer	Buffer	1.16	3.86 p to 386 n	[54]

EIS electrochemical impedance spectroscopy, *DPV* differential pulse voltammetry, *PEC* photoelectrochemical

[a]To calculate concentration, VEGF was assumed to form a dimer

with a dynamic range of approx. 4 pg/ml to 386 ng/ml VEGF, but a proof of concept in serum is missing [54].

2.2.3 Oncolytic Virus

Biosensors are useful not only to detect tumor marker or cancer cells, but also to monitor cancer treatment or to test new treatment strategies. Therapies based on oncolytic viruses might be a game changer for modern cancer treatment. With a special focus on cancer cells, the infection with oncolytic virus causes tumor lysis and supports the development of tumor-specific immunity. Especially promising is this treatment strategy's versatility [55].

However, the immune response might release neutralizing antibodies, shortening the circulation time of oncolytic virus. As a consequence, due to the immunological memory, the application of the oncolytic virus at a later time point is then limited. Impedimetric aptasensors might contribute to overcome this limitation since aptamers as nucleic acids do not activate an immune response. Therefore, aptamers protecting the epitopes can be utilized in order to extend circulation time. Labib et al. proposed an impedimetric aptasensor for testing the degree of protection of an oncolytic virus by different anti-*vesicular stomatitis virus* (VSV) aptamers [56]. In this study, the oncolytic virus VSV was captured by an aptamer immobilized on an electrode surface. After incubation with polyclonal antibodies, the change of impedance determines the degree of virus protection (compare with Fig. 6). The presented aptasensor enabled a linear range of 40,000–110,000 plague forming units (PFU) per ml and a LoD of 30,000 PFU/ml.

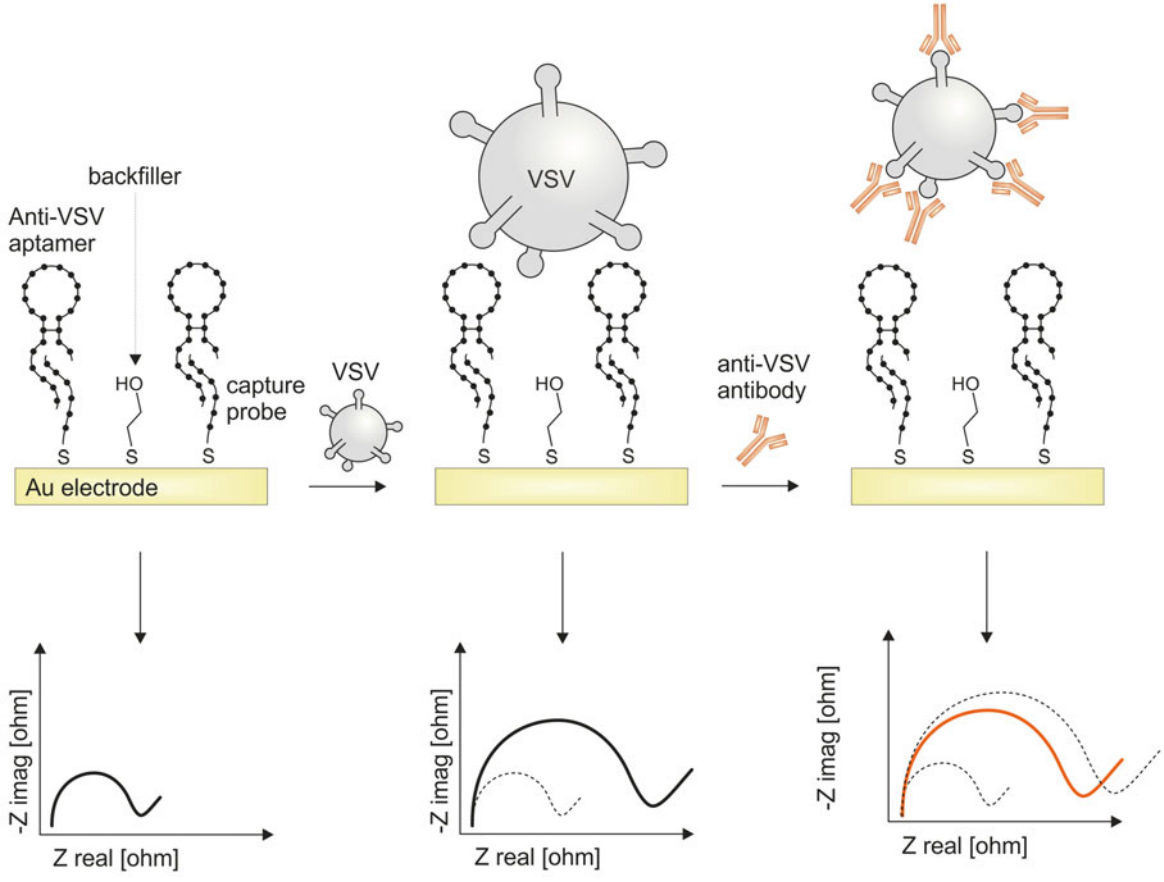


Fig. 6 Impedimetric aptasensor to determine the degree of protection of an oncolytic virus by a specific aptamer with regard to polyclonal antibodies. The approach is proposed as a screening for an aptamer sequence for cancer treatment based on aptamer shielded oncolytic virus injection. (Adapted with permission from [56]. Copyright 2012 American Chemical Society)

Table 5 Different biosensors for endotoxin detection

Method	Biorecognition element	Sample	LoD	Linear range	Reference
EIS	Aptamer	Diluted insulin	7.94 zM[a]	0.01 aM to 1 pM	[59]
EIS	Aptamer	Buffer	5 pg/ml[b]	0.01–10.24 ng/ml	[58]
EIS	Aptamer	Buffer	0.01 ng/ml[c]	0.01–1 ng/ml	[60]
LSPR	Polymyxin B	Buffer	340 pg/ml[d]	10^{-6}–10 μg/ml	[61]
PC-TIR	LAL	LAL reagent water	2.5×10^{-6} EU/ml[e]	2.5×10^{-6} – 5×10^{-2} EU/ml	[57]

LSPR localized surface plasmon resonance, *EIS* electrochemical impedance spectroscopy, *PC-TIR* photonic-crystal total-internal-reflection. For endotoxin, the LoD depends not only on the concentration, but also on the pyrogenicity of the endotoxin that is defined by the endotoxin unit (EU), which varies depending on the source of the endotoxin. Calculations of the endotoxin unit (EU) were based on information about EU and concentrations specified by the author

[a]1 EU/kg body weight = 100 pM

[b]5 pg/ml = 2.5×10^{-3} EU/ml

[c]2 μM = 2×10^{7} EU/ml = 4 mg/ml

[d]Approximately 340 pg/ml = 3 EU/ml

[e]1 EU/ml = 0.1–0.2 ng/ml

2.3 *Other Use Cases*

This paragraph gives a short overview about targets which points beyond the classic diagnostics as a major field of red biotechnology. Since red biotechnology deals with biotechnology in health care related applications in general, the analytics for diverse non-protein or non-cell targets such as drugs are required in order to ensure safe production of pharmaceuticals, drug monitoring, or forensic analytics.

The pyrogenic *endotoxin*, also called *lipopolysaccharide (LPS)*, causes an immune response including fever or septic shock. Therefore, the removal of endotoxins is of great interest for the drug industry concerning potential contamination of pharmaceutical products.

Endotoxins originate from the outer membrane of gram-negative bacteria and are released after cell death and are chemically stable even with regard to sterilization [57, 58]. Thus, the drug industry needs to meet legal limits with regard to endotoxin concentration which lies at approximately 20 pM for drug distribution [59].

The most popular endotoxin detection method is the *Limulus* amebocyte lysate (LAL) test which utilizes the unique coagulation based on an endotoxin induced enzyme cascade in the *Limulus* blood. Chromogenic and turbidimetric assays are available [57]. The need for more rapid testing and the increased need for pharmaceutical contamination detection require more sustainable alternatives than the horseshoe crab based LAL test.

Several impedimetric aptasensors have been developed for the detection of endotoxin. As an example, Posha et al. quantified LPS in diluted, spiked urine with excellent recoveries and a wide linear range from 1 aM to 1 pM, while the lower limit of the range in a PBS buffer system was even 100-fold lower [59]. The surface for aptamer immobilization was increased by gold atomic clusters. In Table 5

biosensors for endotoxin determination are listed, but since EU (endotoxin unit) is a measurement for pyrogenic activity, comparability of different sensor methods is restricted. In all conscience of the announced information by the authors, endotoxin detection limits and ranges are summarized.

Therapeutic drug monitoring (TDM) aims at determining drug concentrations in blood or blood-related samples. Many drugs can be classified as small organic molecules. For the field of affinity-based detection in general, antibodies are the most important strategy. However, the need of immunogenicity usually limits the application of antibodies for small molecule detection [62]. Additionally, antibodies' high molecular mass might reduce its feasibility in many sensor designs with regard to low mass targets. Alternatives such as GC-MS or HPLC require highly trained personnel [63]. Thus, aptamers are considered as an opportunity for drug monitoring and testing.

Medication is sought to be improved by TDM for drugs demanding narrow concentration ranges. Since many drugs are small molecules, the sensors need to be sufficiently sensitive on a small scale. For instance, Roushani and Shahdost-fard presented a sensor for the determination of ibuprofen [64], as the long-term medication risk of ibuprofen with regard to heart attacks is especially striking. Common approaches for its detection are GC-MS or HPLC reaching a LoD of 1 – 8 μM [65, 66]. Roushani and Shahdost-fard reported an ibuprofen detection down to 16 pM with a wide linear range of 50 pM up to 20 μM with high recovery rates in spiked blood. This approach is based on functionalized Quantum dots on an electrode surface. The Quantum dots are supposed to increase the surface and therefore the sensitivity for small molecule determination. The authors immobilized a capture DNA probe on the Quantum dots. The aptamer contains a single-stranded target binding site, while the rest is hybridized on the capture probe for immobilization purposes (see Fig. 7). By a conformational change due to complex formation, an increase of impedance was measured.

2.4 Conclusions

Aptamer-based impedimetric biosensors have been applied to a versatile range of targets underlying the universal qualification as biotechnological tool while the numbers of faradaic EIS applications dominate the non-faradaic ones. From cells to small molecules, the targets of interest generally seem unlimited. Nonetheless, aptamers for further medically relevant targets are required in order to cover the variety of biomarker diagnostics. As a possible alternative to standard techniques, impedimetric aptasensors have been proven to be an easy and rapid diagnostic tool even without elaborate sample preparation due to the label-free application. In many reports, low sample volumes of 5–100 μl were sufficient. A range of authors reported on picomolar detection levels for small molecule and protein targets, while some even reported (sub-)femtomolar LoD levels. Thereby, the success of aptamers as a sensitive recognition element might root in high immobilization densities (approx.

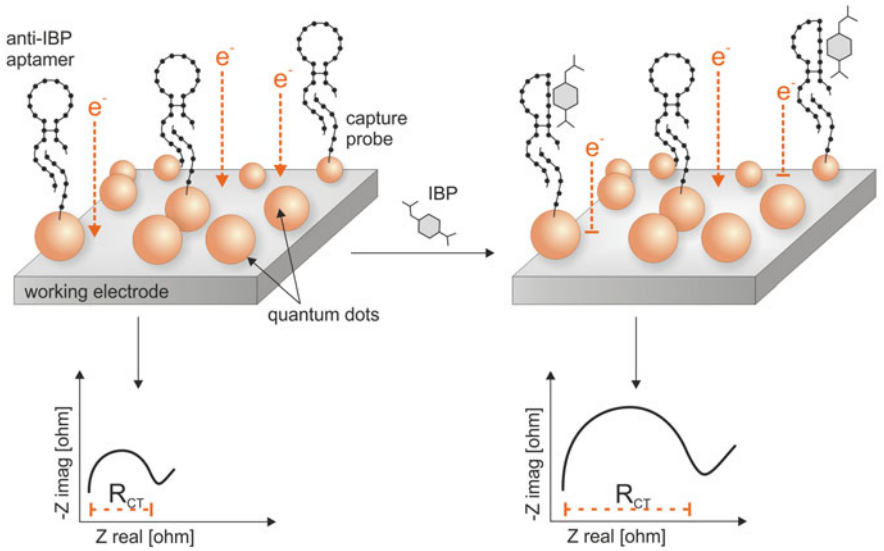


Fig. 7 Ibuprofen (IBP) causes a conformational change of the aptamer resulting in an increase of impedance. A capture probe is immobilized on Quantum dots in order to immobilize the aptamer by hybridization. (Adapted from [64], with permission from Elsevier)

10^{13} aptamer molecules per cm^2) due to its lower molecular mass compared to antibodies [62]. However, for some targets (e.g. lysozyme) no measurement in a medically relevant matrix is reported.

With regard to point-of-care tests, EIS might not be the method of choice since high-priced potentiostats are required and are not suitable for in-field diagnostics (malaria or virus detection). Nonetheless, organizations, which are able to cover the investment costs (e.g., cancer medicine or food industry), might benefit from it by reducing current costs and time. Thus, the important aspects to be clarified are costs per unit, proof of standardized mass production, and tests of stability. The common strategy of self-assembled monolayer formation which requires several hours might be a limiting factor of fabrication and commercialization.

The increase of charge-transfer resistance by a binding event is the most dominant design strategy. An advantage by aptasensors is the relatively low molecular weight of an aptamer compared to an antibody. Therefore, a binding event and the subsequent conformational change might have a higher impact on the charge-transfer.

Another aspect of red biotechnology is the monitoring in biopharmaceutical processes and has not been a topic in literature. To overcome limitations with regard to manual handling which is needed for sample dilution or continuous monitoring, the combination of EIS aptasensors with microfluidic tools might be promising.

3 Impedimetric Aptasensors for Environmental Application (Gray Biotechnology)

In gray biotechnology, impedimetric aptasensors attracted attention in recent years due to Guideline 2008/105/EG of the European Parliament and the Council of 16 December 2008. Gray biotechnology covers biotechnological applications in the environment such as monitoring of drinking water quality and efficiency of wastewater treatment and is therefore also called environmental biotechnology. Impedimetric aptasensors were developed for various purposes in environmental biotechnological applications with the main focus on the detection of pollutants in water sources such as drug residues, bacteria, toxins, and pesticides.

The sources of pollution are versatile, as drug residues are introduced by medical treatments while micropollutants are released into the environment through plastic production. Toxins produced by microorganisms enter the environment during cell death or through their metabolism and accumulate, for example, in shellfish. With impedimetric aptasensors due to the in vitro synthesis of aptamers, it is possible to detect not only proteins, but also toxins, whole cells, and small molecules such as ethanolamine, which has a molecular weight of 61 g/mol and is released into the environment by the textile, chemical, and pharmaceutical industries. In 2016, Liang et al. developed an impedimetric aptasensor for ethanolamine with a detection limit of 0.08 nM, thus more sensitive than currently used chromatographic methods (0.4 μM) [67]. This chapter is intended to give an overview of environmental application of impedimetric biosensors and compares them with classical analytical methods.

3.1 Drug Residues

As mentioned above, drugs are important for medical treatment, but they have significant side effects when they are released into the environment or accumulate in animal products (like milk). The residues of drugs are continuously released into the environment, especially into the water. They can intervene in the regulatory mechanism of the organisms present there. They can be detected there unchanged, since they are often difficult to degrade and mobile. In sewage treatment plants, these can only be retained to a limited extent. Due to the low concentration, many drug residues cannot be detected with the previous methods; therefore, numerous impedimetric aptasensors have been developed for the detection of antibiotics such as tetracycline and chloramphenicol, chemotherapeutics like doxorubicin and hormones such as 17ß-estradiol and progesterone (see Table 6). These drugs are used in cancer therapy, hormone therapy, and animal breeding. With these sensors a faster, simpler, and more sensitive measurement is possible.

Table 6 List of impedimetric aptasensors for the detection of drug residues

Drug residue	Detection limit (M)	Reference
Chloramphenicol	1.76×10^{-9}	[68]
Doxorubicin	2.80×10^{-8}	[69]
	1.28×10^{-7}	[70]
17ß-estradiol	1.00×10^{-15}	[71]
	2.00×10^{-12}	[72]
Progesterone	3.00×10^{-9}	[73]

3.1.1 Antibiotics

Chloramphenicol is an effective broad-spectrum antibiotic used in the treatment of infectious diseases. Due to its potential lethal side effects it was defined that chloramphenicol in any concentration is a health risk. To ensure food safety, the European Commission set a minimum required performance limit (MRPL) value, defining the lowest detectable concentration of the analysis method [74]. Currently, the detection of chloramphenicol is based on gas chromatography, high performance liquid chromatography, and mass spectrometry, which are very sensitive, but are expensive, time-consuming, and require highly trained personnel. Pilehvar et al. developed a simple low-cost aptamer-based impedimetric biosensor with an approximate assay time of 5 h and a detection limit of 1.76 nM (0.6 µg/l), which approaches the MRPL value of 0.3 µg/kg [68].

3.1.2 Anthracycline

Doxorubicin belongs to the group of anthracycline antibiotics and is used as chemotherapeutics in cancer therapy. By incomplete degradation in wastewater, this drug residue reaches our tap water. Due to its cardiotoxicity, it contaminates nature and threatens humans and the environment. An aptamer-based biosensor for detection of doxorubicin by electrochemical impedance spectroscopy was developed by Bahner et al. [69]. The developed sensor was able to reach a detection limit of 28 nM in buffer and thus a lower detection limit as previously reported by Erdem et al. (128 nM) [70].

3.1.3 Hormones

Hormones can also be detected with aptasensors. The hormone estradiol is one of the most important active ingredients for contraception and hormone treatment. When ingested by humans, it can enter the sewage system. Zhu et al. developed a sensitive impedimetric aptasensor for the detection of 17ß-estradiol with a detection limit of 1 fM [71]. They successfully detected estradiol in spiked biologically complex human urine samples as well as spiked untreated tap water samples and obtained

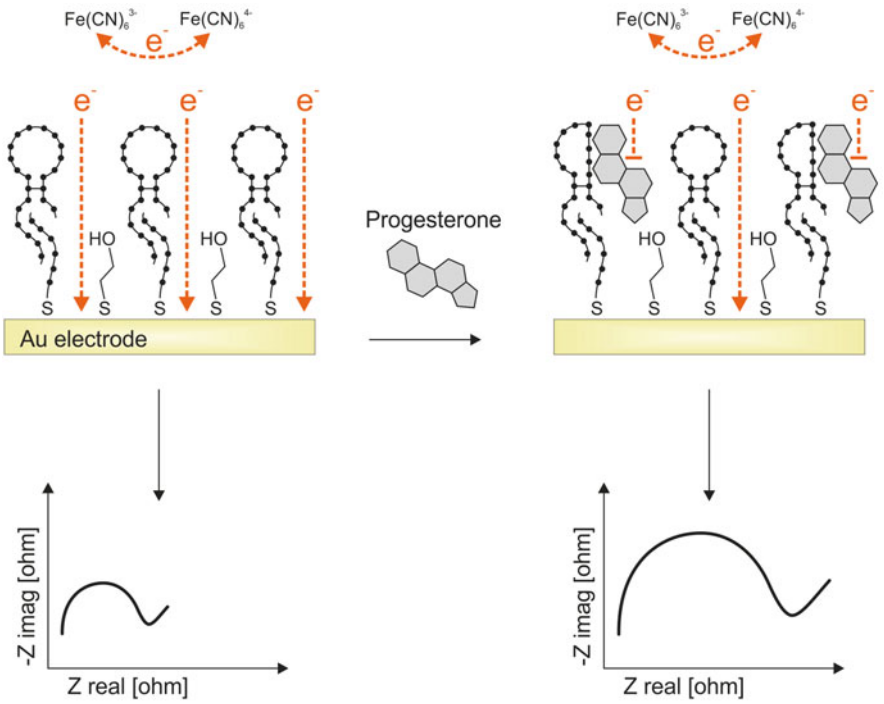


Fig. 8 Principle of an aptamer-based impedimetric biosensor for the detection of progesterone. (Adapted with permission from [73]. Copyright 2015 American Chemical Society)

similar results to estradiol in buffer solution. Also Lin et al. developed in 2012 an aptasensor for the detection of 17ß-estradiol with a detection limit of 2 pM [72]. With the sensor, it was possible to detect 17ß-estradiol in urine, which was added with 17ß-estradiol. Average recovery rates range from 92% to 101% with relative standard deviations below 4.4%. The sensor can therefore be used to determine 17ß-estradiol in urine samples.

Another hormone is progesterone, which is used in hormone therapy. For detection of progesterone in water an aptasensor with a detection limit of 3 nM was developed by Contreras Jimenez et al. (see Fig. 8) [73]. This sensor shows a higher sensitivity than instrumental analysis methods such as HPLC, LC/MS, or GC/MS, because they can detect progesterone concentrations as low as 32 µM.

3.2 Toxins

Toxins produced by bacteria enter the environment during cell death or according to their metabolism. Also the use of insecticides in modern agriculture contaminates the natural environment and through plastic production bisphenol A is released into the

Table 7 List of impedimetric aptasensors for the detection of bacteria and toxins

Analyte	Detection limit	Reference
E. coli O157:H7	100 CFU/ml	[75]
Brevetoxin-2	0.12 nM	[76]
Cylindrospermopsin	0.3 nM	[77]
Microcystin-LR	18 pM	[78]
Atrazine	10 pM	[79]
Bisphenol A	45 fM	[80]
	10 fM	[81]
	7.2 fM	[82]

environment. Therefore, impedimetric aptasensors have been developed for the detection of toxins as the examples shown in Table 7.

3.2.1 *Escherichia coli* and Toxins Produced by Bacteria

One of the main causes of human infectious diseases are pathogenic bacteria such as *Escherichia coli* (*E. coli*). The presence of *E. coli* bacteria in water usually indicates fecal contamination; therefore, a detection of *E. coli* in tap water is required. For routine measurements, a method based on colony forming units is used, which is very sensitive but takes up to 24 h. Thus, biosensors offer a valuable alternative for rapid detection of *E. coli*. For example, the impedimetric aptasensor developed by Brosel-Oliu et al. detects *E. coli* O157:H7 in tap water within 30 min and reaches a detection limit of 100 CFU/ml [75]. Although this sensor is not as sensitive as cultivation methods, it is more sensitive compared to other previously reported biosensors.

Furthermore, microorganisms produce toxins that enter the environment during cell death or through their metabolism. One associated toxin is Brevetoxin-2 that is produced by dinoflagellates *Karenia brevis* and accumulates in shellfish. It has no toxic effect on shellfish, but it is toxic to the sea, mammals, birds, fish, and humans [83]. Eissa et al. developed in 2015 an impedimetric aptasensor against Brevetoxin-2 in shellfish with a detection limit of 0.12 nM [76], which is comparable with previously reported electrochemical immunosensor (0.12 nM) [84].

Cylindrospermopsin, produced by cyanobacteria, is also a kind of widespread toxin in water sources and exhibits strong cytotoxicity and carcinogenic activity [85]. The aptasensor for detection of cylindrospermopsin in lake water has a detection limit of 0.3 nM (0.12 ng/mL) [77] and thus shows a higher sensitivity than instrumental analysis methods such as HPLC or LC/MS that detect cylindrospermopsin up to 1 and 0.5 ng/L [86, 87].

Another toxin produced by cyanobacteria is microcystin-LR (MC-LR), which is released in situation of cell rupture into the water. Due to the toxicity of microcystin-LR, it causes health and environmental problems, e.g. it leads to liver damage and possible death when exposed to humans and animals. Lin et al. developed a sensor

against microcystin-LR and reached a detection limit of 18 pM [78]. Many other methods for MC-LR detection have been reported and showed good detection limits, but the impedimetric aptasensor tops with its simplicity, low-cost, and rapid detection.

3.2.2 Pesticides and Bisphenol A

Pesticides are chemicals that kill fungi, bacteria, insects, snails, slugs, or weed to avoid plant diseases, plant damage, or suppressed growth. Subgroups of pesticides are insecticides, which are substances that are used to kill, harm, repel, or mitigate a specific species of insects. Insecticides are often used to increase crop yields and improve the quality of agricultural products. The use of insecticides in modern agriculture contaminates the natural environment, which can pose a potential health risk to humans. One of the most extensively employed insecticides is acetamiprid that belongs to the class of systemic broad-spectrum insecticides and is used for controlling insects such as *Lepidoptera* and *Thysanoptera* [88]. Aptamer-based biosensors for the detection of acetamiprid are reviewed in Verdian [89] including optical and electrochemical transducers. An impedimetric aptasensor for detection of acetamiprid residues in wastewater and tomatoes was developed by Fan et al. who achieved a detection limit of 1 nM, which is comparable or even lower than conventional instrumental methods (see Table 8) [95]. Fan et al. also examined the application of the sensor for real samples. For this purpose, wastewater samples were added with three different concentrations of acetamiprid and the concentration was determined by the sensor. Average recovery rates range from 94% to 103% with relative standard deviations of 4.9%. The sensor can therefore be used to determine acetamiprid residues in the environment.

Furthermore, an aptamer-based impedimetric biosensor for detection of acetamiprid and atrazine in water was developed [79]. Atrazine is one of the most commonly used herbicides, a subgroup of pesticides used to kill undesirable plants. The developed sensor by Medianos et al. reached a detection limit of 1 pM for acetamiprid and 10 pM for atrazine. In this case, it can be seen that aptasensors allow the detection of molecules in the picomolar concentration range. As far as the

Table 8 Comparison of techniques for the determination of acetamiprid

Method	Detection limit	Reference
HPLC	2.69 nM	[90]
	0.157 nM	[91]
GC	4.49 nM	[92]
	4.49 nM	[93]
ELISA	4.49 nM	[94]
Aptasensor	0.898 nM	[95]
	17 fM	[96]
	33 fM	[97]
	0.9 pM	[79]

detection of acetamiprid is concerned, the proposed impedimetric biosensor is more sensitive than conventional instrumental methods and the aptasensor reported by Fan et al. but lower detection limits of 17–33 fM are reached by other impedimetric biosensors [96, 97]. The developed aptasensor reported the lowest detection limit for atrazine at this time.

Bisphenol A is an organic monomer and is widely used in the chemical industry for manufacturing polycarbonate plastics. In food packaging, it was used, for example, in beverage bottles, baby bottles, and medical devices [98]. Due to its toxicity and harmful endocrine disrupting activity, bisphenol A was banned in baby bottles since 2010 [99]. In recent years, several impedimetric aptasensors against bisphenol A were developed, which allow quick and easy measurement. These sensors reached detection limits of 45 fM [80], 10 fM [81], and 7.2 fM [82], which are the lowest compared to other biosensors.

3.3 An Outlook on Environmental Application of Impedimetric Biosensors

The detection and quantification of pathogens and chemical agents are of great importance for water and environmental analysis. The classical methods such as HPLC and GC/MS are usually time-consuming and require highly qualified personnel and highly developed equipment. Therefore, new technologies are constantly being developed. Aptasensors are real-time measurements of different targets in the environment with high sensitivity and specificity (see Fig. 9).

The aptasensors presented here were able to withstand the interference of complex matrices and can therefore be used for determination in environmental samples. In addition, the detection process with these aptasensors usually only takes 3 h. To improve detection, the aptamers used should be optimized by shortening and stabilizing so that cross sensitivities are reduced. Furthermore, the reproducibility and also the possibility of regeneration must be further investigated.

4 Impedimetric Aptasensors for Food Control

White biotechnology is descriptive for industrial processes to generate products useful for us humans such as chemicals and food. The role of biotechnology in the food industry is copious, ranging from increasing productivity in food manufacturing and handling to fabrication of food additives and preservatives [100]. During growth, fermentation, and processing, the food can be contaminated with microorganisms or toxins. The World Health Organization (WHO) estimated that there are 600 million cases of foodborne diseases every year worldwide. The economic burden of foodborne diseases in Europe is about 171 million US$ per year

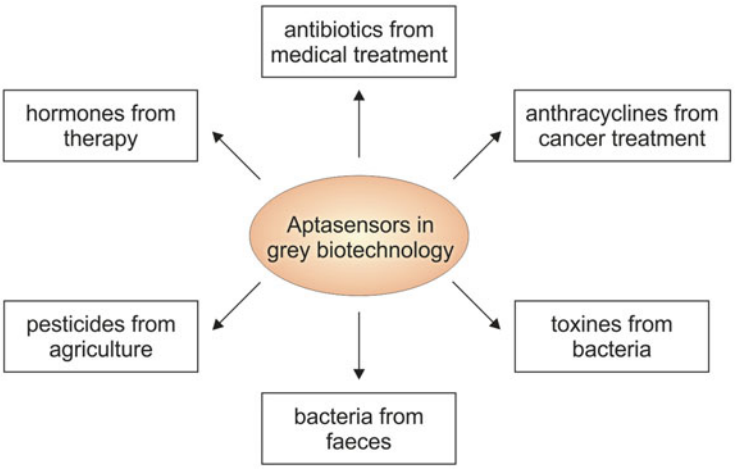


Fig. 9 Overview of the applications of aptasensors in gray biotechnology

[101]. During transportation and storage, the food is densely packed, often still moist and not cooled, which is why microorganisms grow and produce toxins (see Fig. 10). Thus, food control upon arrival at the factory as well as before the delivery to the consumer is essential. Especially small manufacturers have a high demand in compact devices with easy handling for rapid on-site detection. Beside ELISAs and microarrays, biosensors are an alternative for rapid detection of contaminants. An important step in food control is the extraction of the analytes from the food matrix which might be reduced in biosensor setups by the use of stable and biocompatible recognition elements, such as aptamers. In this chapter, we will review the advances in aptamer-based impedimetric biosensors for the detection of mycotoxins and microorganisms in food.

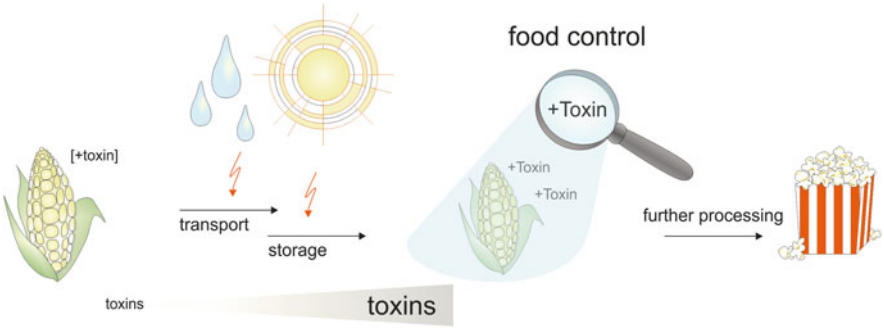


Fig. 10 Role of food control in white biotechnology – it is essential to control food before processing at the factory – methods for fast detection in situ are desired, as e.g. biosensors

4.1 Mycotoxins

Mycotoxins are metabolites produced by fungi that are toxic for humans and animals. Fungi grow best in moisture and warm environments and thus, mycotoxins are found in products grown in hot climates or stored and shipped for long periods such as coffee beans or peanuts. The toxins withstand high temperatures and thus remain active after heat treatment. Furthermore, mycotoxins do not induce an immune response and thus are bypassing our immune system. To produce antibodies against a mycotoxin, it needs to be bound to an immunogenic protein carrier which is cumbersome. Therefore, especially for small molecules like mycotoxins, aptamers are a perfect alternative recognition element in biosensors. The major groups of mycotoxins are aflatoxin, ochratoxin, citrinin, ergot alkaloids, patulin, and fusarium [102]. Vidal et al. published a review discussing electrochemical biosensors for the detection of mycotoxins in food and they conclude that label-free and direct binding properties, as impedimetric biosensors have, are beneficial in quality control in food processing [103]. A list of aptamer-based impedimetric biosensors for the detection of mycotoxins is presented in Table 9.

Aflatoxins are produced mainly by *Aspergillus flavus*, *A. parasiticus,* and *A. nomius*, contaminants found in nuts and grains. Not only concentrations of ~10 μg/kg body weight have an acute hepatotoxic effect, but also concentrations below are carcinogenic [102]. From 20 known types of aflatoxins, aflatoxin B_1 (AFB1) is considered the most prevalent, followed by aflatoxin B_2, G_1, and G_2. The European Commission set the limit for AFB1 in food products containing nuts and grains depending on the kind and handling to 2–12 ppb (equivalent to 2–12 ng/g or 6.4–38.4 nM) and for processed food intended for babies or children to 0.1 ng/g (equivalent to 0.32 nM) [116].

Castillo et al. developed an aptamer-based impedimetric biosensor for the detection of AFB1 by using PAMAM G4 dendrimers on gold electrodes to increase the

Table 9 Impedimetric aptasensors for the detection of mycotoxins

Toxin	Conc. range in g/ml	LoD in pg/ml	Real sample	Response time	Reference
OTA	100 p to 25 m	100	–	15 min	[104]
OTA	40 p to 40 n	50	Coffee, flour, wine	30 min	[105]
OTA	120 p to 12 n	20	Beer	30 min	[106]
OTA	1 p to 0.5 n	0.2	Beer	1 h	[107]
OTA	4–40 p	6	White wine	90 min	[108]
OTA	2 p to 6 n	2	Wine	50 min	[109]
OTA	0.01 f to 0.1 n	10^{-5}	Soybean	9 min	[110]
OTA	0.1–10 p	0.05	Grape juice	5–7 min	[111]
AFB1	31 p to 2 n	100	Peanuts	40 min	[112]
AFB1	1 f to 1 n	0.4×10^{-3}	Peanuts	3 h	[113]
AFM1	20 p to 1 n	1	Milk	30 min	[114]
FB1	72 p to 72 m	1	Maize	30 min	[115]

active surface ([112], see Fig. 11). As the pK of AFB1 is 5.84, the impedance increased upon its binding to the immobilized aptamers. An affinity constant K_d of 0.59 ± 0.33 nM and a limit of detection (LoD) of 0.4 ± 0.03 nM in buffer were measured, proving high affinity and sensitivity. Slight interferences with aflatoxin B_2 were observed, but no interference with ochratoxin A. Measurements of certified contaminated peanut extracts resulted in recovery rates of 96–120%, but in spiked peanut samples matrix effects were discovered. The developed biosensor has an assay time of 30 min, is regenerable with 0.2 mM glycine, and is stable for 60 h when stored at 4°C. As a rule of thumb, the detection limit should be ~5 times below the legislative limit, thus the detection range is sufficient for the food control of nuts and grains for the direct human consumption but not for baby food. As comparison, the LoD of other developed biosensors were in the range from 0.1 to 6.4 nM, but some researchers claimed lower limits, e.g. Li et al. who achieved a LoD of 1.3 fM by aptamer-based surface enhanced Raman spectroscopy (SERS) and signal enhancement by a recycling mechanism [113]. However, the impedimetric aptasensor developed by Castillo et al. is 4–5 times faster and thus offers a cost-effective alternative to ELISAs for small food manufacturers.

The presence of AFB1 in feed leads to its digestion in lactating animals producing its hydroxylated metabolites aflatoxin M_1 (AFM1) and M_2. Thus, the EU regulated the concentration of AFM1 in raw milk to 0.05 µg/kg (equivalent to 152 nM) and in milk-based products intended for infants to 0.025 µg/kg [116]. Istamboulié et al. developed a faradaic impedance biosensor using screen-printed carbon electrodes

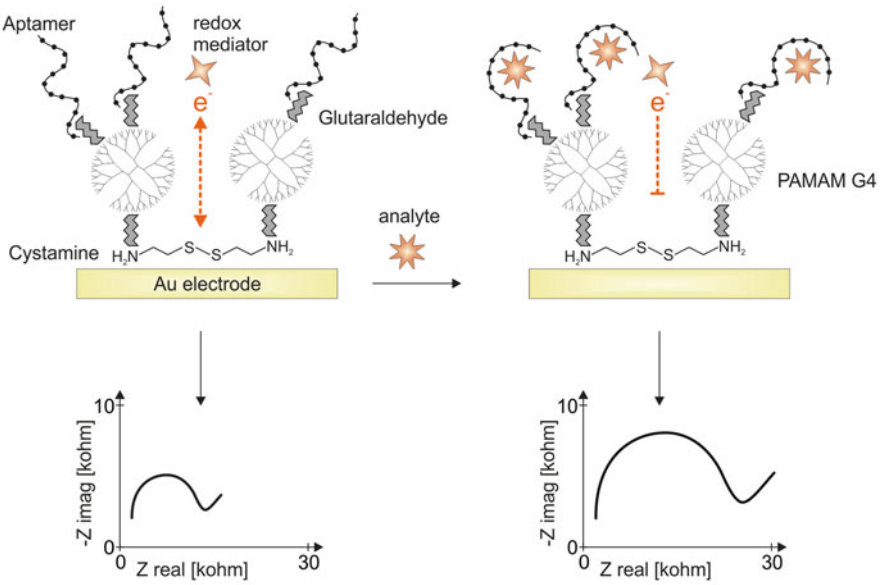


Fig. 11 Modification of the surface with the dendrimer PAMAM G4 increases the active surface and thus enables signal enhancement. (Adapted from [112], with permission from Elsevier)

(SPCE) covered with 4-carboxyphenyl diazonium salt (4-CP) and 5′-amino-hexaethyleneglycol modified aptamers against AFM1 [114]. They reached in buffer a LoD of 1.15 ng/l and in milk a linear range of 20–1,000 ng/kg. Measurements of raw milk and pasteurized milk spiked with 500 ng/kg AFM1 resulted in recovery rates of 99–111%, whereas measurements with a standard immunoassay resulted in similar recoveries of 98–114%. The comparison to a standard method proves its functionality, although interferences with other substances should be tested.

Hianik's research group immobilized aptamers against AFM1 on the PAMAM G4 modified electrodes and compared them to biotin-avidin surfaces [117]. The results demonstrate that both immobilization methods show the same characteristics with a dynamic range of 15–120 ng/l and a LoD of 8.5 ng/l in milk which is sufficient for the legislative limits.

Ochratoxin is produced by fungi of the genera *Aspergillus* and *Penicillium*. It can be found in cereals, coffee, dried fruits, cocoa, grapes, and spices as well as in processed food like red wine, bread, beverages and it can be accumulated in animals, e.g. pork meat. Ochratoxin A (OTA) is the most prevalent and relevant representative of the ochratoxins and has several toxicological effects such as neurotoxicity, nephropathy, immunosuppression, and carcinogenicity [118]. The European Commission set regulatory limits of OTA in foodstuff depending on the process level from 2 to 20 ng/g and for foodstuff intended for babies or infants to 0.5 ng/g [116].

Castillo et al. from Hianik's research group developed an aptamer-based impedimetric biosensor for the detection of OTA by simple chemisorption of the aptamers on a gold electrode and measuring the impedance in buffer with 1 mM of the redox mediator ferri-/ferrocyanide $[Fe(CN)_6]^{3-/4-}$ [105]. They tested different modifications of the aptamer for immobilization; interestingly, the simplest modification with a thiol on the 5′ end showed the best results reaching a LoD of 0.12 nM (=0.05 ng/ml) and they notified that Ca^{2+} ions were essential for OTA binding. They also measured the surface density of aptamers and obtained 1.85×10^{13} aptamers/cm^2. Furthermore, OTA showed a six times higher signal than OTB and *N*-Acetyl-L-phenylalanine. The recoveries in coffee, flour, and wine spiked with 1, 5, and 10 ng/g OTA were 78–108%. The sensor could also be regenerated by immersion into 1 mM HCl and no significant decrease of the signal was observed within 10 cycles of regeneration.

Evtugyn et al. from the same research group used silver nanoparticles to enhance the signal and obtained a LoD of 0.05 nM (=0.02 ng/ml) for OTA in buffer and a concentration range of 0.3–30 nM [106], although they observed instability of the coating and used significant higher concentrations of $[Fe(CN)_6]^{3-/4-}$ (0.1 M instead of 1 mM).

For the direct detection in situ, biosensors with a wide concentration range are favored. Hayat et al. developed an aptamer-based impedimetric biosensor for the detection of OTA with a concentration range of 1.25–500 ng/l and a LoD of 0.25 ng/l [107]. This low sensitivity was achieved by the modification of a SPCE with diazonium salts and the immobilization of the aptamer via click chemistry. The surface modification led to high impedances of about 150 kΩ and thus low frequencies of 10 mHz were required to measure the charge-transfer resistance in a buffer

solution with 1 mM $[Fe(CN)_6]^{3-/4-}$. Although the author enabled a wide concentration range, it is two magnitudes below the legislative limits and thus a dilution step is required. The concentration range achieved by Castillo et al. of 0.04–40 ng/ml is better suited for direct detection [105]. However, dilution of the sample can increase the signal-to-noise ratio as the matrix is diluted as well. The authors were able to regenerate the aptasensor up to 10 times without significant signal loss.

A wide detection range can be obtained by increasing the active-surface-to-volume ratio as Mejri-Omrani et al. achieved by modifying a gold electrode with a conductive polypyrrole layer (PPy) and above a layer of dendrimers (PAMAM G4) containing a high number of immobilization sites for the aptamer (see Fig. 12, [109]). They measured a detection range from 2 pg/ml to 6 ng/ml, which is similar to the ranges measured with high pressure liquid chromatography (HPLC), but shifted to a lower detection limit [119]. The lower concentrations reached by Mejri-Omrani et al. compared to Castillo et al. for AFB1 [112] may be grounded in a higher affinity of the aptamer, the higher concentration of the redox mediator $[Fe(CN)_6]^{3-/4-}$ (10 mM) or it may be due to the addition of the PPy layer. However, the median concentration of OTA in wine is 50 pg/g and thus the demonstrated aptasensor is a promising alternative for OTA detection in wine.

An astonishing wide concentration range and detection limit of 0.01 fg/ml to 0.1 ng/ml was achieved by Wei et al. by using a nanocomposite of porous carbon structures with incorporated gold nanoparticles which significantly increases the

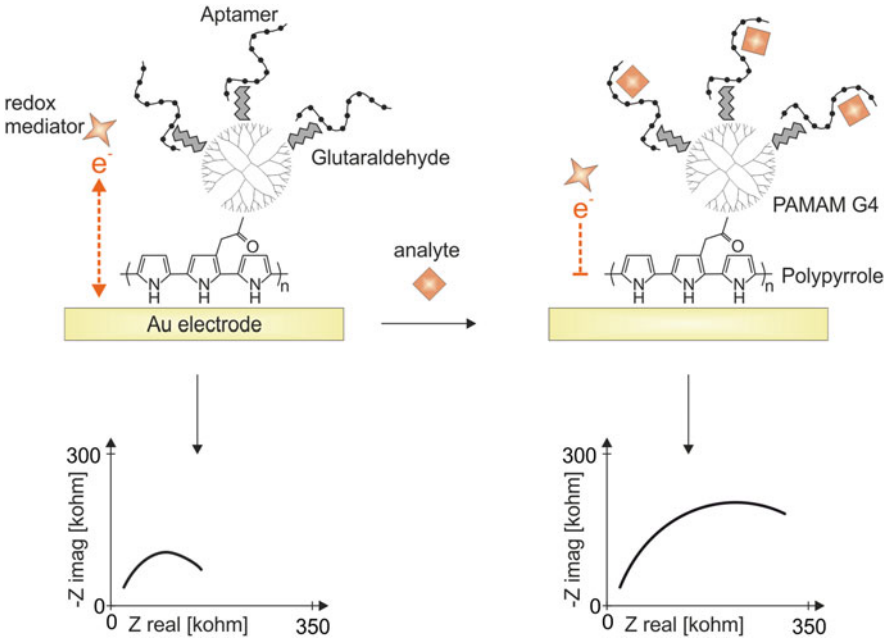


Fig. 12 Signal enhancement by modification of the surface with the dendrimer PAMAM G4 and the conductive polymer polypyrrole (PPy). (Adapted from [109], with permission from Elsevier)

active surface [110]. The achieved detection limit is even lower than the LoD obtained with real-time PCR (1 fg/ml), although such sensitivities are not necessary for the application in food control. The developed aptasensor was tested on soybean samples and recoveries of 95–108% were obtained. Compared to the simple sensor surface of Castillo's aptasensor [105], the signal ratio of OTA to AFB1 is only ~2.5. It is reasonable to presume that by increasing the active surface, the unspecific binding is increased as well.

In conclusion, impedimetric aptasensors for mycotoxin detection are well-developed, providing detection limits and ranges comparable with HPLC and sufficient for the legislative limits set by the EC. They were validated with real samples and complex food matrices with short measurement times of 5–90 min and have mechanisms for regeneration. For the transfer of these biosensors into commercial products, handheld impedance analyzers with microfluidic cartridges are needed.

4.2 Microorganisms

Besides mycotoxins from fungi, another major cause of foodborne diseases are the toxins produced by bacteria like *Salmonella, Escherichia coli, Staphylococcus aureus,* and *Bacillus cereus*. Although it is more accurate to directly determine the toxins, often in food control the microorganisms are detected.

Optical biosensors for the detection of pathogenic bacteria are reviewed in Yoo and Lee [120], whereas electrochemical biosensors are reviewed by Amiri et al. [121] who concluded that aptamers, imprinted polymers, and bacteriophages are promising alternatives to antibodies and that improvements are needed in reproducibility and reliability as well as in minimizing non-specific binding. In comparison with clinical applications that require spotting of a single bacterium, in food control higher concentrations are sufficient, as for example 10^5 colony forming units (CFU) per ml of *Staphylococcus aureus* (*S. aureus*) are producing toxins in a quantity that can cause food poisoning. A general review of biosensors for the detection of foodborne pathogens is given by Arora et al. [122], whereas Teng et al. [123] reviewed aptamer-based biosensors and Kant et al. [124] concentrate on microfluidic devices. In this chapter, we will review aptamer-based impedimetric biosensors for the detection of food borne pathogens (see Table 10).

Salmonella are gram-negative, flagellated, and facultative anaerobic bacteria from the family Enterobacteriaceae. More than 2,600 serotypes are distinguished by the existence of different antigens, like the somatic O-antigen, also known as lipopolysaccharide or endotoxin (see Sect. 2.3) and the flagellar H antigen. However, the most common serovar found in humans and animals are *S. typhimurium* and *S. enteritidis* that also the major pathogens in gastrointestinal infections. Salmonella food poisoning is mainly caused not only by contaminated poultry and eggs, but also by the feces of infected humans and animals, insufficient hygiene in food handling, or contaminated surface water. Thus, the EC regulation No. 2073/2005 demands the

Table 10 Impedimetric aptasensors for the detection of microorganisms and their toxins in food

Analyte	Method	LoD in CFU/ml	Real sample	Incubation time in min	Reference
S. typhimurium	EIS	600	–	60	[125]
S. typhimurium	EIS	25	Chicken meat	60	[126]
S. typhimurium	EIS	6	Apple juice	60	[127]
S. typhimurium	EIS	3	Apple juice	45	[128]
S. typhimurium	EIS	3	Pork meat	35	[129]
S. typhimurium	EIS	1	Egg	40	[130]
S. typhimurium	CA	20	Chicken meat	120	[131]
S. typh. and *E. coli*	RT-PCR	78	Milk	120	[132]
S. tnteritidis	EIS	600	–	60	[133]
E. coli	EIS	2×10^4	–	60	[134]
E. coli	EIS	4	–	12	[135]
E. coli	EIS	12	Milk	120	[136]
Listeria	EIS	100	–	<180	[137]
L. monocytogenes	EIS	5	–	17	[138]
S. aureus	EIS	10	Fish and water	60	[139]
S. aureus	EIS	10	–	10	[140]
SEB	EIS	6 pM	Milk	100	[141]

absence of Salmonella in 25 g of meat, gelatin, collagen, milk products, eggs, and many kinds of ready-to-eat products including sea food, fruits, and vegetables [142].

Ma et al. developed an aptamer-based faradaic impedance biosensor for the detection of *S. typhimurium* by modifying a glassy carbon electrode with a nanocomposite of graphene oxide and gold nanoparticles [129]. They reached a detection limit (LoD) of 3 colony forming units (CFU) per ml in buffer and a linear range from 2.4 to 2.4×10^3 CFU/ml with a sensitivity of 706 Ohm per decade. The high specificity of the biosensor was shown with a 12 times higher signal for *S. typhimurium* compared to *L. monocytogenes*, *B. subtilis*, *S. aureus*, *S. pyogenes*, *E. coli,* and *Enterobacter sakazakii*. Performance was verified in spiked pork samples with recovery rates of 97–105%. With an incubation time of 35 min, the whole measurement can be performed within 40–50 min, which is significantly faster than a real-time PCR, taking 2 h and reaching a LoD of 78 CFU/ml [132].

An impressing detection limit of 1 CFU/ml was achieved by Ranjibar et al. by nanoporous gold electrodes modified with thiolated aptamers [130]. The developed impedimetric aptasensor has a wide dynamic range (6.5×10^2–6.5×10^8 CFU/ml) with a sensitivity of 841 Ohm per decade and a high selectivity with a 7 times higher signal for *S. typhimurium* compared to *S. aureus*, *E. coli,* and *Pseudomonas*. Recovery rates from spiked egg samples were 85–110% and the RSD from repeated

experiments was only 1.47%. With an incubation time of 40 min, this biosensor represents an attracting alternative for routinely food control purposes.

At last, we want to report on the biosensor developed by Sheikhzadeh et al. as this is a truly label-free impedimetric aptasensor that does not require any redox probe but enables faradaic measurements by the use of a conductive polypyrrole copolymer [128]. They reached a LoD of 3 CFU/ml and a wide linear range from 10^2 to 10^8 CFU/ml. The biosensor showed good selectivity (150%) and repeatability (5.2%, each concentration was repeated three times), but performed poorly on spiked apple juice samples with recovery rates of 140–410%.

Escherichia coli is a bacteria that is found in the intestines of humans and most types are harmless, but some cause food borne diseases such as the Shiga toxin producing *E. coli* (STEC). According to EFSA (European Food Safety Authority), there was an outbreak of STEC in Germany in 2011 that caused ~4,000 cases of bloody diarrhea and hemolytic uremic syndrome. The EC sets limits for relevant foodborne bacteria in products placed on the market and defines process hygiene criteria to ensure food safety [142]. To this regulation, STEC should be absent in 25 g of sprouts placed on the market during their shelf-life. Besides, the detection of *E. coli* is used as an indicator for fecal contamination, products with low hygiene or high microbial contamination.

Burrs et al. [135] developed an impedimetric biosensor based on a RNA aptamer that specifically binds to the O-antigen on the cell surface of the STEC stem O157: H7 [143]. They increased the electroactive surface of graphene paper by functionalization with fractal platinum nanocauliflower and obtained a roughness factor R (= geometrical area/ electroactive surface) of 10. Immobilized thiolated aptamers enabled the binding of *E. coli* from the sample resulting in an increase of impedance. They achieved a LoD of 4 CFU/ml and a linear range from 4 to 10^5 CFU/ml with a response time of 12 min by a low cost of 4 $ per sample. However, the developed aptasensor needs to be verified in real samples with complex matrices as no prior sample preparation step is intended.

Dua et al. developed a non-faradaic impedance biosensor based on a 2′-fluoro-pyrimidines modified RNA aptamer selected by Cell-SELEX against *E. coli* DH5α [134]. They used a gap sensor of two gold electrodes modified with the aptamers with a distance of 4 μm in a microfluidic channel. The sample is incubated for 1 h resulting not only in an increase of the impedance at low frequencies representing the solution resistance, but also in an increase of the impedance at high frequencies (10 kHz). With this sensor a LoD of 104 CFU/ml was achieved.

Yao et al. were able to detect bacteria concentrations as low as 12 CFU/ml by a procedure combining antibody-modified magnetic nanoparticles for sample preparation and aptamer-modified gold nanoparticles with immobilized urease [136]. With the addition of the substrate urea, ammonium carbonate is produced. The supernatant of the enzymatic reaction will be transferred into a microfluidic cell with interdigitated microelectrodes and the impedance at 15 kHz is measured every 2 s. The impedance decreases with increasing bacteria concentration due to the higher conductivity of the product (see Fig. 13). The procedure was verified with spiked pasteurized milk. This procedure contains a lot of premeasurement steps,

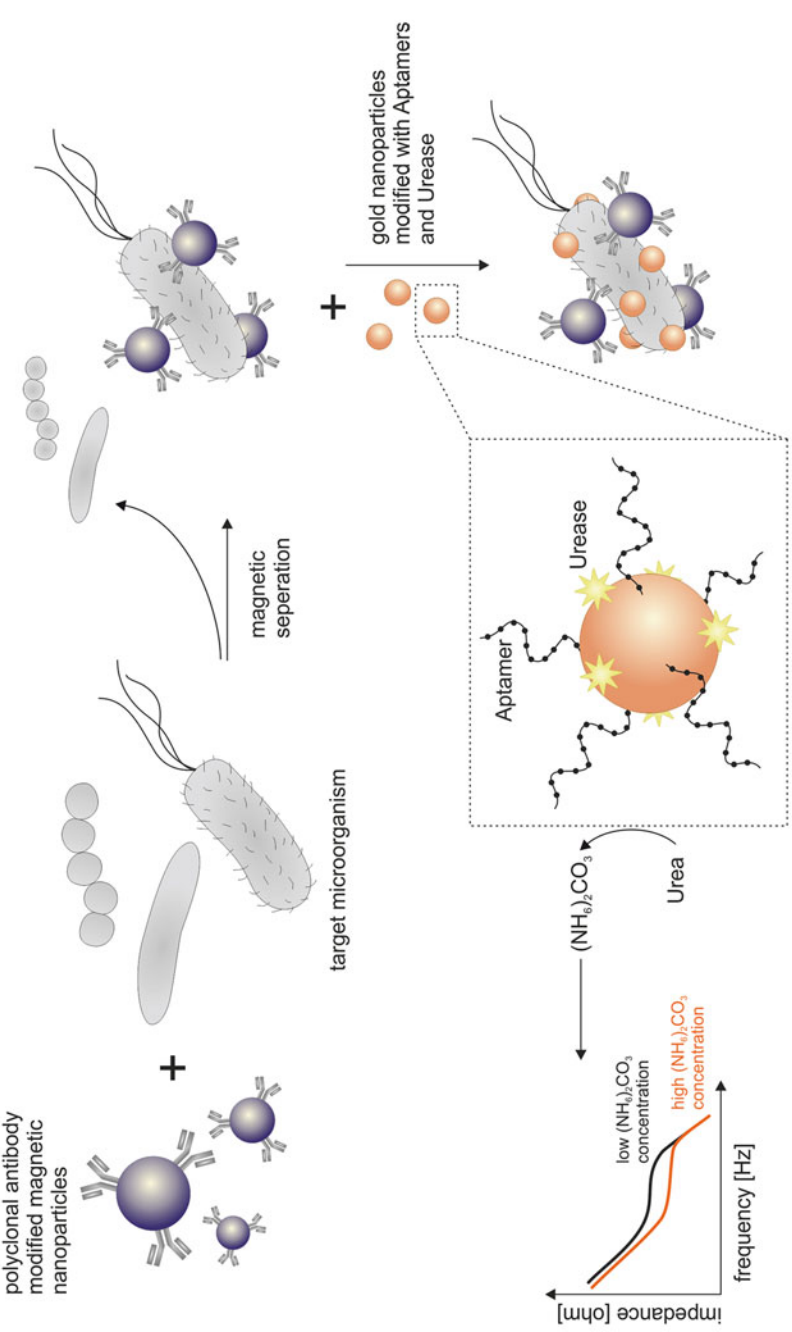


Fig. 13 Detection of microorganisms by magnetic separation and enzymatic conversion of urea. Urease modified gold nanoparticles are labeled with aptamers to specifically detect desired microorganism. Upon addition of urea the impedance decreases proportionally. (Adapted from [136], with permission of Elsevier)

which enable a 5 times concentration of the bacteria but taking all together around 2 h which is hampering the development of point-of-care tests. However, purification steps will be necessary as the different ionic strength of samples will complicate the measurement.

Listeria, *Staphylococci,* and *Enterobacteriaceae* are also listed in the EC regulation [142]. There are two publications on aptamer-based impedimetric biosensors for the detection of *Listeria* reaching LoDs of 100 CFU/ml [137] and 5 CFU/ml [138] but without verification on real samples.

Rubab et al. published a review about biosensors for the detection of *S. aureus* in food, which is a main representative of *Staphylococci* in food poisoning due to the production of several exo- and enterotoxins such as *staph.* enterotoxin A and B [144]. However, an ingestion of 144 ng *staph.* enterotoxin B (SEB) can cause gastroenteritis with symptoms as vomiting and diarrhea [145]. Thus, the EC postulates the absence of *staph.* enterotoxins in 25 g cheese, milk powder, and whey powder.

Xiong et al. developed an aptamer-based impedimetric biosensor for the sensitive detection of SEB as low as 0.17 ng/ml by the simple modification of gold electrodes with thiolated aptamers and mercaptohexanol as backfiller [141]. They could detect SEB in a linear range from 0.5 to 500 ng/ml within 100 min and proved a high selectivity with a 9 times higher signal for SEB compared to other toxins found in food such as OTA, SEA, and AFB1. Furthermore, they verified the biosensor in spiked milk samples and obtained recovery rates of 77–119%, which were compared to measurements with an ELISA kit resulting in relative deviations of −8 to 8%.

The EC also demands a limit of coagulase-positive *Staphylococci* that is 10 CFU/g in different cheese products, milk powder, and whey powder. The coagulase-test is used to differentiate between toxic strains like *S. aureus* and harmless strains like *S. epidermidis*, which are coagulase-negative. Jia et al. developed an aptamer-based impedimetric biosensor for the detection of *S. aureus* by using a nanocomposite of reduced graphene and gold nanoparticles to increase the effective surface [139]. They reached a detection of 10 CFU/ml within 60 min and verified the performance in fish and water samples with recovery rates of 92–114%. Reich et al. performed impedimetric detection of *S. aureus* in buffer using aptamers immobilized on a gold electrode in a flow-through chamber [140]. They reached a LoD of 10 CFU/ml within 10 min, which is significantly faster than most other methods, but they did not show any verification in real samples.

In conclusion, the development of impedimetric aptasensors for food control has advanced in recent years. The reviewed biosensors showed sufficient detection limits and proved functionality in real samples. However, most are still time-consuming taking several hours and thus offer no advantage over ELISA and PCR. Although by miniaturization of the electrochemical setups, space saving and cost-efficient solutions will be developed in the near future.

5 Benefits and Challenges of Impedimetric Aptasensors

In the first sections of this chapter, we reviewed aptamer-based impedimetric biosensors with respect to their application in biotechnology. An advantage of electrochemical biosensors is their high sensitivity and in combination with aptamers and surface design by using, e.g., nanocomposites or other nanostructures, the detection limit dropped below 10 fM in the last years (see Table 11). Posha et al. reached the impressive detection limit of 0.01 aM which is only one decade above the single molecule detection in 1 ml of sample (1.7 zM). Enabling single molecule detection would make calibrations redundant and thus, spare valuable time and efforts. Also for the detection of bacteria, detection limit of 4 CFU/ml [135] and 5 CFU/ml [138] were achieved which is close to single cell detection.

As presented in this review, there is a huge variety of surface modifications from single monolayers and porous electrodes to complex nanocomposites. Most surface modifications aim to increase the effective surface which may also increase the unspecific binding, as e.g. seen in [149], leading to a loss of sensitivity. Thus, the challenge in surface engineering is to increase the effective surface and simultaneously decrease unspecific binding. When measuring in real samples, low unspecific binding is important, as seen in [150], where due to unspecific interactions a quantification of the analyte in serum was not possible. The reduction of unspecific binding enables measurement in complex sample matrices without the need of purification steps. Most of the reviewed articles are measuring the calibration curve in buffer solutions and validations of the sensor are performed in undiluted simpler matrices such as urine [72, 151], extracted food matrices [110, 126], or water [77, 80]. But for complex matrices such as blood, serum, and milk, the validation is mostly performed in filtered or diluted samples. Some exceptions exist, for example

Table 11 Impedimetric aptasensors with detection limits below 10 fM

Analyte	LoD	Real sample	Surface modification	Reference
Endotoxin	0.01 aM	Insulin	Au atomic cluster	[59]
OTA	25 aM	Soybean	AuNPs porous carbon	[110]
PSA	27 aM	Serum	TiO_2-silk fibroin nanofiber-composite	[146]
Heparanase	52 aM	–	Hydrogel with cDNA	[147]
17ß-estradiol	1 fM	Tap water, urine	Nanoporous electrode	[71]
Thrombin	1 fM	Blood, Cerebro-spinal fluid	TiO_2-NPs/MWCNT/ chitosan-nanocomposite	[23]
PDGF-BB	2.4 fM	–	$Co_3(PO_4)_2$-nanocomposite	[148]
AFM1	3.5 fM	Filtered milk	Diazonium salt	[114]
Tetracycline	3.8 fM	Drug, milk, honey, serum	Magnetic bar carbon paste/Fe_3O_4-NPs/Oleic acid	[149]
CEA	5 fM	Serum	Mesoporous silica film	[43]
Bisphenol A	7.2 fM	Milk	AuNPs + boron-doped diamond (BDD)	[82]

Lee et al. who successfully validated the developed aptasensor for the detection of malaria by *Plasmodium* lactate dehydrogenase (pLDH) with infected blood samples from human patients [31] and Shi et al. who used serum samples of cancer patients to validate the biosensor for ATP detection [152]. We found 47 other impedimetric aptasensors (most covered in this chapter) that could detect the analyte in serum samples, which show the tendency of real sample validation. However, more clinical sample validation experiments are needed. Key parameters that should be tested in real samples are repeatability, non-specific binding, and sample to sample variation. The performance of aptasensors in real sample settings could be improved by adjusting the buffer during SELEX to mimic real samples.

The SELEX procedure has been developed substantially in the last decade. Still, the intrinsic capability to engineer an aptamer adapted to the specific needs of the application has not been fully utilized. During the SELEX process several steps can be included to reduce non-specific binding [153, 154] and to include a regeneration technique [155]. For example, Wehbe et al. included an EDTA wash step to obtain switchable aptamers (SwAps) that were used to recover vesicular stomatitis virus after capture to allow further analytics of the sample with recovery rates of ~33%. This technique would allow performing several analyses on the same sample for, e.g., lab-on-a-chip developments.

Further strategies for the regeneration of the aptamer-modified sensors were developed, mostly based on chemicals changing the ionic strength or pH of the media, e.g. 2 M NaCl [156], 6 M guanidine hydrochloride [157], 6 M urea [32], pH 4 [158], 0.2 M glycine-HCl [112], or imidazole [159]. Others use more complex elution buffers, e.g. Tris buffer containing 20% of methanol [107] or mixtures of different chemicals, e.g. 0.05 M NaOH and 1 M NaCl [160] or variations of several properties, like 1 M NaOH at 4°C [45]. But also more complex mechanisms for the regeneration of the aptamer-target-bond were developed, e.g. the abovementioned SwAps or the usage of mixed aptamers (MBA) that are oligonucleotides containing more than one aptamer sequence directed against different targets. Du et al. used a combination of ATP and thrombin aptamer, whereas the MBA was immobilized by a complementary DNA probe immobilized on the electrode surface [161]. Part of the ATP aptamer was complement to the DNA probe leading to a release of the MBA when it binds to ATP. The binding of thrombin did not lead to a release but to an increase in impedance. Thus, this aptasensor can be used to detect thrombin and can be regenerated with ATP. A similar technique used by Liu et al. to detect Mucin 1 protein and carcinoembryonic antigen [51] is hybridization of the aptamer to an immobilized complementary oligonucleotide, where upon binding to its target the aptamer is released and the biosensor can be easily regenerated.

Detection of only one marker is not enough to establish a reliable diagnosis, but biosensors targeting multiple analytes would enable early detection and help doctors to provide a personalized therapy [162]. Although several publications report about micro- and nanoarrays of electrodes [163–166] that are suitable for multiplexing including repetitions and controls, unfortunately, no report on impedimetric aptasensor used for the simultaneous detection of multiple analytes was found. Multiplex impedimetric aptasensors would significantly enhance the chance for market penetration and thus more research in this direction is needed.

For the development of handheld point-of-care tests, microfluidic chips need to be established for the usage with aptamer-based impedimetric biosensors. Some preliminary work was done by [167–169] who used microelectrodes on glass substrate and fixed a molded microfluidic channel from polydimethylsiloxane (PDMS) on the electrodes. The use of microfluidics in impedimetric biosensors reduces the amount of sample and enables low-cost disposable biosensors. Additionally, different mixing mechanisms can be included to accelerate the reaction-diffusion kinetics [170], as for example used by Feng et al. who reduced the response time from 60 min to only 15 min by magnetic stirring [171].

As impedimetric biosensors are valued for their fast detection, several impedimetric aptasensors with analysis times below 15 min were developed, as e.g. for OTA in 15, 9, or 5 min [104, 110, 111], for *E. coli* within 12 min [135] and for *S. aureus* in 10 min [140]. If, instead of a whole spectrum, the signal at a single frequency is measured, the response time is below ms and real-time monitoring can be performed. Liao et al. developed a non-faradaic impedance aptasensor measuring at 5 kHz and thus, enabling in vivo monitoring of PDGF-BB [172].

Another issue is the stability of aptamers, as RNA and DNA aptamers are sensitive to nucleases abundant in biological fluids leading to their degradation within minutes [173]. Thus modified nucleotides were developed to increase nuclease-resistance, such as 2′-fluoro- and 2′-amino-pyrimidines or 2′-hydroxyl-purines, which led to the first clinically approved aptamer known as Macugen® [174]. González-Fernández et al. developed an impedimetric biosensor using a fully 2′-O-methylated aptamer and detected tobramycin in diluted human serum [175]. Other possibilities to increase the stability of aptamers are 3′- or 5′-end cappings, locked amino acids [LNA], or peptide nucleic acids [PNA]. The aptamer can be modified post-SELEX, but this often leads to a significant decrease in affinity [176]. However, any modification on the aptamer increases its production costs and may imply complications within synthesis. Thus, more research is still needed.

All over, we found electrochemical sensors based on aptamers for about 100 different targets and the number is rising. The thrombin aptamer is best examined, but little is known about the mechanism of aptamer-target binding and how it is influenced by its surrounding, such as pH, ions, and temperature. Beside experiments, more mathematical calculation and simulation of electrical properties are needed.

Impedimetric aptasensors will not supersede well-established methods like PCR, ELISA, or HPLC, but rather fill an important gap as e.g. for the detection of small non-immunogenic molecules. With the advances in nanotechnology, enabling the creation of nanostructures, it is likely that we will soon be able to detect single molecules, especially in combination with handling of nanoliter volumes. This will require sensor miniaturization, as e.g. with integrated microelectronics, for what impedimetric aptasensors are optimally suited. With the already established variety of surfaces, impedimetric aptasensors can be customized for many applications in biotechnology, especially for extreme conditions such as high temperatures, high pH, and complex biological matrices. Nevertheless, it is important to advance the

basic research on aptamer-target binding and develop models to enable effective biosensor design.

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References

1. Jo H, Kim SK, Youn H, Lee H, Lee K, Jeong J, Mok J, Kim SH, Park HS, Ban C (2016) A highly sensitive and selective impedimetric aptasensor for interleukin-17 receptor A. Biosens Bioelectron 81:80–86. https://doi.org/10.1016/j.bios.2016.02.050
2. Ocaña C, del Valle M (2014) Signal amplification for thrombin impedimetric aptasensor: sandwich protocol and use of gold-streptavidin nanoparticles. Biosens Bioelectron 54:408–414. https://doi.org/10.1016/j.bios.2013.10.068
3. Qi H, Shangguan L, Li C, Li X, Gao Q, Zhang C (2013) Sensitive and antifouling impedimetric aptasensor for the determination of thrombin in undiluted serum sample. Biosens Bioelectron 39(1):324–328. https://doi.org/10.1016/j.bios.2012.07.040
4. Snyderman R (2012) Personalized health care: from theory to practice. Biotechnol J 7 (8):973–979. https://doi.org/10.1002/biot.201100297
5. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC, Taubert K, Tracy RP, Vinicor F (2003) Markers of inflammation and cardiovascular disease application to clinical and public health practice – a statement for healthcare professionals from the centers for disease control and prevention and the American Heart Association. Circulation 107(3):499–511. https://doi.org/10.1161/01.Cir.0000052939.59093.45
6. Qureshi A, Gurbuz Y, Kallempudi S, Niazi JH (2010) Label-free RNA aptamer-based capacitive biosensor for the detection of C-reactive protein. Phys Chem Chem Phys 12 (32):9176–9182. https://doi.org/10.1039/c004133e
7. Piccoli J, Hein R, El-Sagheer AH, Brown T, Cilli EM, Bueno PR, Davis JJ (2018) Redox capacitive assaying of C-reactive protein at a peptide supported aptamer interface. Anal Chem 90(5):3005–3008. https://doi.org/10.1021/acs.analchem.7b05374
8. Aray A, Chiavaioli F, Arjmand M, Trono C, Tombelli S, Giannetti A, Cennamo N, Soltanolkotabi M, Zeni L, Baldini F (2016) SPR-based plastic optical fibre biosensor for the detection of C-reactive protein in serum. J Biophotonics 9(10):1077–1084. https://doi.org/10.1002/jbio.201500315
9. Bryan T, Luo XL, Bueno PR, Davis JJ (2013) An optimised electrochemical biosensor for the label-free detection of C-reactive protein in blood. Biosens Bioelectron 39(1):94–98. https://doi.org/10.1016/j.bios.2012.06.051
10. Bing X, Wang G (2017) Label free C-reactive protein detection based on an electrochemical sensor for clinical application. Int J Electrochem Sci 12(7):6304–6314
11. Wu B, Jiang R, Wang Q, Huang J, Yang XH, Wang KM, Li WS, Chen ND, Li Q (2016) Detection of C-reactive protein using nanoparticle- enhanced surface plasmon resonance using an aptamer-antibody sandwich assay. Chem Commun 52(17):3568–3571. https://doi.org/10.1039/c5cc10486f
12. Zubiate P, Zamarreno CR, Sanchez R, Matias IR, Arregui FJ (2017) High sensitive and selective C-reactive protein detection by means of lossy mode resonance based optical fiber devices. Biosens Bioelectron 93:176–181. https://doi.org/10.1016/j.bios.2016.09.020
13. Vasilescu A, Wang Q, Li MS, Boukherroub R, Szunerits S (2016) Aptamer-based electrochemical sensing of lysozyme. Chemosensors 4(2):20. https://doi.org/10.3390/chemosensors4020010

14. Peng Y, Zhang D, Li Y, Qi H, Gao Q, Zhang C (2009) Label-free and sensitive faradic impedance aptasensor for the determination of lysozyme based on target-induced aptamer displacement. Biosens Bioelectron 25(1):94–99. https://doi.org/10.1016/j.bios.2009.06.001
15. Xia Y, Gan S, Xu Q, Qiu X, Gao P, Huang S (2013) A three-way junction aptasensor for lysozyme detection. Biosens Bioelectron 39(1):250–254. https://doi.org/10.1016/j.bios.2012.07.053
16. Zhang Z, Zhang S, He L, Peng D, Yan F, Wang M, Zhao J, Zhang H, Fang S (2015) Feasible electrochemical biosensor based on plasma polymerization-assisted composite of polyacrylic acid and hollow TiO_2 spheres for sensitively detecting lysozyme. Biosens Bioelectron 74:384–390. https://doi.org/10.1016/j.bios.2015.06.062
17. Chen ZB, Li LD, Zhao HT, Guo L, Mu XJ (2011) Electrochemical impedance spectroscopy detection of lysozyme based on electrodeposited gold nanoparticles. Talanta 83(5):1501–1506. https://doi.org/10.1016/j.talanta.2010.11.042
18. Khan NI, Maddaus AG, Song E (2018) A low-cost inkjet-printed aptamer-based electrochemical biosensor for the selective detection of lysozyme. Biosensors 8(1):7. https://doi.org/10.3390/bios8010007
19. Dong YP, Wang J, Peng Y, Zhu JJ (2017) A novel aptasensor for lysozyme based on electrogenerated chemiluminescence resonance energy transfer between luminol and silicon quantum dots. Biosens Bioelectron 94:530–535. https://doi.org/10.1016/j.bios.2017.03.044
20. Krenzlin H, Lorenz V, Danckwardt S, Kempski O, Alessandri B (2016) The importance of thrombin in cerebral injury and disease. Int J Mol Sci 17(1):84. https://doi.org/10.3390/ijms17010084
21. Deng CY, Chen JH, Nie LH, Nie Z, Yao SZ (2009) Sensitive bifunctional aptamer-based electrochemical biosensor for small molecules and protein. Anal Chem 81(24):9972–9978. https://doi.org/10.1021/ac901727z
22. Lu L, Li J, Kang T, Cheng S (2015) Bi-functionalized aptasensor for ultrasensitive detection of thrombin. Talanta 138:273–278. https://doi.org/10.1016/j.talanta.2015.03.016
23. Heydari-Bafrooei E, Amini M, Ardakani MH (2016) An electrochemical aptasensor based on TiO_2/MWCNT and a novel synthesized Schiff base nanocomposite for the ultrasensitive detection of thrombin. Biosens Bioelectron 85:828–836. https://doi.org/10.1016/j.bios.2016.06.012
24. Gaffen SL (2009) Structure and signalling in the IL-17 receptor family. Nat Rev Immunol 9(8):556–567. https://doi.org/10.1038/nri2586
25. Tertiş M, Ciui B, Suciu M, Săndulescu R, Cristea C (2017) Label-free electrochemical aptasensor based on gold and polypyrrole nanoparticles for interleukin 6 detection. Electrochim Acta 258:1208–1218. https://doi.org/10.1016/j.electacta.2017.11.176
26. Jo H, Gu H, Jeon W, Youn H, Her J, Kim SK, Lee J, Shin JH, Ban C (2015) Electrochemical Aptasensor of cardiac troponin I for the early diagnosis of acute myocardial infarction. Anal Chem 87(19):9869–9875. https://doi.org/10.1021/acs.analchem.5b02312
27. Boriani G, Biffi M, Cervi V, Bronzetti G, Magagnoli G, Zannoli R, Branzi A (2000) Evaluation of myocardial injury following repeated internal atrial shocks by monitoring serum cardiac troponin I levels. Chest 118(2):342–347. https://doi.org/10.1378/chest.118.2.342
28. Wang B, Jing R, Qi HL, Gao Q, Zhang CX (2016) Label-free electrochemical impedance peptide-based biosensor for the detection of cardiac troponin I incorporating gold nanoparticles modified carbon electrode. J Electroanal Chem 781:212–217. https://doi.org/10.1016/j.jelechem.2016.08.005
29. Akter R, Jeong B, Lee YM, Choi JS, Rahman MA (2017) Femtomolar detection of cardiac troponin I using a novel label-free and reagent-free dendrimer enhanced impedimetric immunosensor. Biosens Bioelectron 91:637–643. https://doi.org/10.1016/j.bios.2017.01.021
30. Jorgensen P, Chanthap L, Rebueno A, Tsuyuoka R, Bell D (2006) Malaria rapid diagnostic tests in tropical climates: the need for a cool chain. Am J Trop Med Hyg 74(5):750–754. https://doi.org/10.4269/ajtmh.2006.74.750

31. Lee S, Song KM, Jeon W, Jo H, Shim YB, Ban C (2012) A highly sensitive aptasensor towards plasmodium lactate dehydrogenase for the diagnosis of malaria. Biosens Bioelectron 35 (1):291–296. https://doi.org/10.1016/j.bios.2012.03.003
32. Figueroa-Miranda G, Feng L, Shiu SC-C, Dirkzwager RM, Cheung Y-W, Tanner JA, Schöning MJ, Offenhäusser A, Mayer D (2018) Aptamer-based electrochemical biosensor for highly sensitive and selective malaria detection with adjustable dynamic response range and reusability. Sensors Actuators B Chem 255:235–243. https://doi.org/10.1016/j.snb.2017.07.117
33. Kiilerich-Pedersen K, Daprà J, Cherré S, Rozlosnik N (2013) High sensitivity point-of-care device for direct virus diagnostics. Biosens Bioelectron 49:374–379. https://doi.org/10.1016/j.bios.2013.05.046
34. Otero C, Penaloza JP, Rodas PI, Fernandez-Ramires R, Velasquez L, Jung JE (2014) Temporal and spatial regulation of cAMP signaling in disease: role of cyclic nucleotide phosphodiesterases. Fundam Clin Pharmacol 28(6):593–607. https://doi.org/10.1111/fcp.12080
35. Zhao F, Xie Q, Xu M, Wang S, Zhou J, Liu F (2015) RNA aptamer based electrochemical biosensor for sensitive and selective detection of cAMP. Biosens Bioelectron 66:238–243. https://doi.org/10.1016/j.bios.2014.11.024
36. Wang Y, Feng J, Tan Z, Wang H (2014) Electrochemical impedance spectroscopy aptasensor for ultrasensitive detection of adenosine with dual backfillers. Biosens Bioelectron 60:218–223. https://doi.org/10.1016/j.bios.2014.04.022
37. Feng L, Chen Y, Ren J, Qu X (2011) A graphene functionalized electrochemical aptasensor for selective label-free detection of cancer cells. Biomaterials 32(11):2930–2937. https://doi.org/10.1016/j.biomaterials.2011.01.002
38. Kashefi-Kheyrabadi L, Mehrgardi MA, Wiechec E, Turner AP, Tiwari A (2014) Ultrasensitive detection of human liver hepatocellular carcinoma cells using a label-free aptasensor. Anal Chem 86(10):4956–4960. https://doi.org/10.1021/ac500375p
39. Hashkavayi AB, Raoof JB, Ojani R, Kavoosian S (2017) Ultrasensitive electrochemical aptasensor based on sandwich architecture for selective label-free detection of colorectal cancer (CT26) cells. Biosens Bioelectron 92:630–637. https://doi.org/10.1016/j.bios.2016.10.042
40. Shen H, Yang J, Chen Z, Chen X, Wang L, Hu J, Ji F, Xie G, Feng W (2016) A novel label-free and reusable electrochemical cytosensor for highly sensitive detection and specific collection of CTCs. Biosens Bioelectron 81:495–502. https://doi.org/10.1016/j.bios.2016.03.048
41. Tsuchiya N, Sawada Y, Endo I, Saito K, Uemura Y, Nakatsura T (2015) Biomarkers for the early diagnosis of hepatocellular carcinoma. World J Gastroenterol 21(37):10573–10583. https://doi.org/10.3748/wjg.v21.i37.10573
42. Laboria N, Fragoso A, Kemmner W, Latta D, Nilsson O, Botero ML, Drese K, O'Sullivan CK (2010) Amperometric immunosensor for carcinoembryonic antigen in colon cancer samples based on monolayers of dendritic bipodal scaffolds. Anal Chem 82(5):1712–1719. https://doi.org/10.1021/ac902162e
43. Shekari Z, Zare HR, Falahati A (2017) Developing an impedimetric aptasensor for selective label–free detection of CEA as a cancer biomarker based on gold nanoparticles loaded in functionalized mesoporous silica films. J Electrochem Soc 164(13):B739–B745. https://doi.org/10.1149/2.1991713jes
44. Wang W, Ge L, Sun X, Hou T, Li F (2015) Graphene-assisted label-free homogeneous electrochemical biosensing strategy based on aptamer-switched bidirectional DNA polymerization. ACS Appl Mater Interfaces 7(51):28566–28575. https://doi.org/10.1021/acsami.5b09932
45. Guo C, Su F, Song Y, Hu B, Wang M, He L, Peng D, Zhang Z (2017) Aptamer-templated silver nanoclusters embedded in zirconium metal-organic framework for bifunctional electrochemical and SPR aptasensors toward carcinoembryonic antigen. ACS Appl Mater Interfaces 9(47):41188–41199. https://doi.org/10.1021/acsami.7b14952

46. Wang K, He MQ, Zhai FH, He RH, Yu YL (2017) A novel electrochemical biosensor based on polyadenine modified aptamer for label-free and ultrasensitive detection of human breast cancer cells. Talanta 166:87–92. https://doi.org/10.1016/j.talanta.2017.01.052
47. Zhou F, Yuan L, Wang H, Li D, Chen H (2011) Gold nanoparticle layer: a promising platform for ultra-sensitive cancer detection. Langmuir 27:2155–2158. https://doi.org/10.1021/la1049937
48. Qureshi A, Gurbuz Y, Niazi JH (2015) Capacitive aptamer–antibody based sandwich assay for the detection of VEGF cancer biomarker in serum. Sensors Actuators B Chem 209:645–651. https://doi.org/10.1016/j.snb.2014.12.040
49. Tabrizi MA, Shamsipur M, Farzin L (2015) A high sensitive electrochemical aptasensor for the determination of VEGF(165) in serum of lung cancer patient. Biosens Bioelectron 74:764–769. https://doi.org/10.1016/j.bios.2015.07.032
50. Salven P, Perhoniemi V, Tykkä H, Mäenpää H, Joensuu H (1999) Serum VEGF levels in women with a benign breast tumor or breast cancer. Breast Cancer Res Treat 53(2):161–166
51. Liu C, Liu X, Qin Y, Deng C, Xiang J (2016) A simple regenerable electrochemical aptasensor for the parallel and continuous detection of biomarkers. RSC Adv 6(63):58469–58476. https://doi.org/10.1039/C6RA09284E
52. Teke M, Sayikli C, Canbaz C, Sezgintürk MK (2014) A novel biosensing system using biological receptor for analysis of vascular endothelial growth factor. Int J Pept Res Ther 20 (2):221–230. https://doi.org/10.1007/s10989-013-9386-4
53. Pan LH, Kuo SH, Lin TY, Lin CW, Fang PY, Yang HW (2017) An electrochemical biosensor to simultaneously detect VEGF and PSA for early prostate cancer diagnosis based on graphene oxide/ssDNA/PLLA nanoparticles. Biosens Bioelectron 89:598–605. https://doi.org/10.1016/j.bios.2016.01.077
54. Da HM, Liu HY, Zheng YN, Yuan R, Chai YQ (2018) A highly sensitive VEGF(165) photoelectrochemical biosensor fabricated by assembly of aptamer bridged DNA networks. Biosens Bioelectron 101:213–218. https://doi.org/10.1016/j.bios.2017.10.032
55. Achard C, Surendran A, Wedge ME, Ungerechts G, Bell J, Ilkow CS (2018) Lighting a fire in the tumor microenvironment using oncolytic immunotherapy. EBioMedicine 31:17–24. https://doi.org/10.1016/j.ebiom.2018.04.020
56. Labib M, Zamay AS, Muharemagic D, Chechik A, Bell JC, Berezovski MV (2012) Electrochemical sensing of aptamer-facilitated virus immunoshielding. Anal Chem 84(3):1677–1686. https://doi.org/10.1021/ac202978r
57. Scudder J, Ye JY (2018) Limulus amoebocyte lysate test via an open-microcavity optical biosensor. J Biomed Opt 23(2):6. https://doi.org/10.1117/1.Jbo.23.2.027001
58. Su W, Kim SE, Cho M, Nam JD, Choe WS, Lee Y (2013) Selective detection of endotoxin using an impedance aptasensor with electrochemically deposited gold nanoparticles. Innate Immun 19(4):388–397. https://doi.org/10.1177/1753425912465099
59. Posha B, Nambiar SR, Sandhyarani N (2018) Gold atomic cluster mediated electrochemical aptasensor for the detection of lipopolysaccharide. Biosens Bioelectron 101:199–205. https://doi.org/10.1016/j.bios.2017.10.030
60. Kim SE, Su W, Cho M, Lee Y, Choe WS (2012) Harnessing aptamers for electrochemical detection of endotoxin. Anal Biochem 424(1):12–20. https://doi.org/10.1016/j.ab.2012.02.016
61. Zandieh M, Hosseini SN, Vossoughi M, Khatami M, Abbasian S, Moshaii A (2018) Label-free and simple detection of endotoxins using a sensitive LSPR biosensor based on silver nanocolumns. Anal Biochem 548:96–101. https://doi.org/10.1016/j.ab.2018.02.023
62. Walter JG, Heilkenbrinker A, Austerjost J, Timur S, Stahl F, Scheper T (2012) Aptasensors for small molecule detection. ZNaturforsch(B) 67(10):976–986. https://doi.org/10.5560/znb.2012-0147
63. Roushani M, Shahdost-Fard F (2018) Impedimetric detection of cocaine by using an aptamer attached to a screen printed electrode modified with a dendrimer/silver nanoparticle nanocomposite. Microchim Acta 185(4):8. https://doi.org/10.1007/s00604-018-2709-6

64. Roushani M, Shandost-fard F (2017) Ultra-sensitive detection of ibuprofen (IBP) by electrochemical aptasensor using the dendrimer-quantum dot (Den-QD) bioconjugate as an immobilization platform with special features. Mater Sci Eng C Mater Biol Appl 75:1091–1096. https://doi.org/10.1016/j.msec.2017.03.023
65. Alsirawan MB, Mohammad M, Al-kasmi B, Alhareth K, El-Hammadi M (2013) Development and validation of a simple HPLC method for the determination of ibuprofen sticking onto punch faces. Int J Pharm Pharm Sci 5(Suppl 4):227–231
66. Stephenson JB, Flater ML, Bain LT (2016) Analysis of Valproic acid, salicylic acid and ibuprofen in whole blood by GC-MS. J Anal Toxicol 40(8):649–652. https://doi.org/10.1093/jat/bkw079
67. Liang G, Man Y, Jin X, Pan L, Liu X (2016) Aptamer-based biosensor for label-free detection of ethanolamine by electrochemical impedance spectroscopy. Anal Chim Acta 936:222–228. https://doi.org/10.1016/j.aca.2016.06.056
68. Pilehvar S, Dierckx T, Blust R, Breugelmans T, De Wael K (2014) An electrochemical impedimetric aptasensing platform for sensitive and selective detection of small molecules such as chloramphenicol. Sensors 14(7):12059–12069. https://doi.org/10.3390/s140712059
69. Bahner N, Reich P, Frense D, Menger M, Schieke K, Beckmann D (2018) An aptamer-based biosensor for detection of doxorubicin by electrochemical impedance spectroscopy. Anal Bioanal Chem 410(5):1453–1462. https://doi.org/10.1007/s00216-017-0786-8
70. Erdem A, Karadeniz H, Caliskan A (2011) Dendrimer modified graphite sensors for detection of anticancer drug Daunorubicin by voltammetry and electrochemical impedance spectroscopy. Analyst 136(5):1041–1045. https://doi.org/10.1039/c0an00357c
71. Zhu B, Alsager OA, Kumar S, Hodgkiss JM, Travas-Sejdic J (2015) Label-free electrochemical aptasensor for femtomolar detection of 17beta-estradiol. Biosens Bioelectron 70:398–403. https://doi.org/10.1016/j.bios.2015.03.050
72. Lin Z, Chen L, Zhang G, Liu Q, Qiu B, Cai Z, Chen G (2012) Label-free aptamer-based electrochemical impedance biosensor for 17beta-estradiol. Analyst 137(4):819–822. https://doi.org/10.1039/c1an15856b
73. Contreras Jiménez G, Eissa S, Ng A, Alhadrami H, Zourob M, Siaj M (2015) Aptamer-based label-free impedimetric biosensor for detection of progesterone. Anal Chem 87(2):1075–1082. https://doi.org/10.1021/ac503639s
74. Commission Regulation (EC) No 181/2003 (2003) 2003/181/EC: commission decision of 13 March 2003 amending decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin
75. Brosel-Oliu S, Ferreira R, Uria N, Abramova N, Gargallo R, Muñoz-Pascual F-X, Bratov A (2018) Novel impedimetric aptasensor for label-free detection of *Escherichia coli* O157:H7. Sensors Actuators B Chem 255:2988–2995. https://doi.org/10.1016/j.snb.2017.09.121
76. Eissa S, Siaj M, Zourob M (2015) Aptamer-based competitive electrochemical biosensor for brevetoxin-2. Biosens Bioelectron 69:148–154. https://doi.org/10.1016/j.bios.2015.01.055
77. Zhao Z, Chen H, Ma L, Liu D, Wang Z (2015) A label-free electrochemical impedance aptasensor for cylindrospermopsin detection based on thionine-graphene nanocomposites. Analyst 140(16):5570–5577. https://doi.org/10.1039/c5an00704f
78. Lin Z, Huang H, Xu Y, Gao X, Qiu B, Chen X, Chen G (2013) Determination of microcystin-LR in water by a label-free aptamer based electrochemical impedance biosensor. Talanta 103:371–374. https://doi.org/10.1016/j.talanta.2012.10.081
79. Madianos L, Tsekenis G, Skotadis E, Patsiouras L, Tsoukalas D (2018) A highly sensitive impedimetric aptasensor for the selective detection of acetamiprid and atrazine based on microwires formed by platinum nanoparticles. Biosens Bioelectron 101:268–274. https://doi.org/10.1016/j.bios.2017.10.034
80. Azadbakht A, Roushani M, Abbasi AR, Derikvand Z (2016) A novel impedimetric aptasensor, based on functionalized carbon nanotubes and prussian blue as labels. Anal Biochem 512:58–69. https://doi.org/10.1016/j.ab.2016.08.006

81. Kazane I, Gorgy K, Gondran C, Spinelli N, Zazoua A, Defrancq E, Cosnier S (2016) Highly sensitive bisphenol-A electrochemical aptasensor based on poly(Pyrrole-Nitrilotriacetic acid)-aptamer film. Anal Chem 88(14):7268–7273. https://doi.org/10.1021/acs.analchem.6b01574
82. Ma Y, Liu J, Li H (2017) Diamond-based electrochemical aptasensor realizing a femtomolar detection limit of bisphenol A. Biosens Bioelectron 92:21–25. https://doi.org/10.1016/j.bios.2017.01.041
83. Flewelling LJ, Naar JP, Abbott JP, Baden DG, Barros NB, Bossart GD, Bottein M-YD, Hammond DG, Haubold EM, Heil CA, Henry MS, Jacocks HM, Leighfield TA, Pierce RH, Pitchford TD, Rommel SA, Scott PS, Steidinger KA, Truby EW, Van Dolah FM, Landsberg JH (2005) Red tides and marine mammal mortalities. Nature 435:755. https://doi.org/10.1038/nature435755a
84. Tang D, Tang J, Su B, Chen G (2011) Gold nanoparticles-decorated amine-terminated poly (amidoamine) dendrimer for sensitive electrochemical immunoassay of brevetoxins in food samples. Biosens Bioelectron 26(5):2090–2096. https://doi.org/10.1016/j.bios.2010.09.012
85. Bazin E, Huet S, Jarry G, Hégarat LL, Munday JS, Humpage AR, Fessard V (2012) Cytotoxic and genotoxic effects of cylindrospermopsin in mice treated by gavage or intraperitoneal injection. Environ Toxicol 27(5):277–284. https://doi.org/10.1002/tox.20640
86. Eaglesham GK, Norris RL, Shaw GR, Smith MJ, Chiswell RK, Davis BC, Neville GR, Seawright AA, Moore MR (1999) Use of HPLC-MS/MS to monitor cylindrospermopsin, a blue–green algal toxin, for public health purposes. Environ Toxicol 14(1):151–154. https://doi.org/10.1002/(SICI)1522-7278(199902)14:1<151::AID-TOX19>3.0.CO;2-D
87. Guzmán-Guillén R, Prieto AI, González AG, Soria-Díaz ME, Cameán AM (2012) Cylindrospermopsin determination in water by LC-MS/MS: optimization and validation of the method and application to real samples. Environ Toxicol Chem 31(10):2233–2238. https://doi.org/10.1002/etc.1954
88. Seccia S, Fidente P, Barbini DA, Morrica P (2005) Multiresidue determination of nicotinoid insecticide residues in drinking water by liquid chromatography with electrospray ionization mass spectrometry. Anal Chim Acta 553(1):21–26. https://doi.org/10.1016/j.aca.2005.08.006
89. Verdian A (2018) Apta-nanosensors for detection and quantitative determination of acetamiprid – A pesticide residue in food and environment. Talanta 176:456–464. https://doi.org/10.1016/j.talanta.2017.08.070
90. Tanner G, Czerwenka C (2011) LC-MS/MS analysis of neonicotinoid insecticides in honey: methodology and residue findings in Austrian honeys. J Agric Food Chem 59 (23):12271–12277. https://doi.org/10.1021/jf202775m
91. Dujaković N, Grujić S, Radišić M, Vasiljević T, Laušević M (2010) Determination of pesticides in surface and ground waters by liquid chromatography–electrospray–tandem mass spectrometry. Anal Chim Acta 678(1):63–72. https://doi.org/10.1016/j.aca.2010.08.016
92. Zhang B, Pan X, Venne L, Dunnum S, McMurry ST, Cobb GP, Anderson TA (2008) Development of a method for the determination of 9 currently used cotton pesticides by gas chromatography with electron capture detection. Talanta 75(4):1055–1060. https://doi.org/10.1016/j.talanta.2008.01.032
93. Mateu-Sánchez M, Moreno M, Arrebola FJ, Martínez Vidal JL (2003) Analysis of acetamiprid in vegetables using gas chromatography-tandem mass spectrometry. Anal Sci 19(5):701–704. https://doi.org/10.2116/analsci.19.701
94. Wanatabe S, Ito S, Kamata Y, Omoda N, Yamazaki T, Munakata H, Kaneko T, Yuasa Y (2001) Development of competitive enzyme-linked immunosorbent assays (ELISAs) based on monoclonal antibodies for chloronicotinoid insecticides imidacloprid and acetamiprid. Anal Chim Acta 427(2):211–219. https://doi.org/10.1016/S0003-2670(00)01126-0
95. Fan L, Zhao G, Shi H, Liu M, Li Z (2013) A highly selective electrochemical impedance spectroscopy-based aptasensor for sensitive detection of acetamiprid. Biosens Bioelectron 43:12–18. https://doi.org/10.1016/j.bios.2012.11.033
96. Fei A, Liu Q, Huan J, Qian J, Dong X, Qiu B, Mao H, Wang K (2015) Label-free impedimetric aptasensor for detection of femtomole level acetamiprid using gold nanoparticles decorated

multiwalled carbon nanotube-reduced graphene oxide nanoribbon composites. Biosens Bioelectron 70:122–129. https://doi.org/10.1016/j.bios.2015.03.028
97. Jiang D, Du X, Liu Q, Zhou L, Dai L, Qian J, Wang K (2015) Silver nanoparticles anchored on nitrogen-doped graphene as a novel electrochemical biosensing platform with enhanced sensitivity for aptamer-based pesticide assay. Analyst 140(18):6404–6411. https://doi.org/10.1039/c5an01084e
98. Wang W, Yang X, Gu Y-X, Ding C-F, Wan J (2015) Preparation and properties of bisphenol A sensor based on multiwalled carbon nanotubes/$Li_4Ti_5O_{12}$-modified electrode. Ionics 21 (3):885–893. https://doi.org/10.1007/s11581-014-1217-x
99. Commission Regulation (EU) No 10/2011 (2011) Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food
100. Ghoshal G (2018) Chapter 2 – biotechnology in food processing and preservation: an overview. In: Holban AM, Grumezescu AM (eds) Advances in biotechnology for food industry. Academic Press, Cambridge, pp 27–54. https://doi.org/10.1016/B978-0-12-811443-8.00002-5
101. Kuchenmüller T, Hird S, Stein C, Kramarz P, Nanda A, Havelaar AH (2009) Estimating the global burden of foodborne diseases – a collaborative effort. Eur Secur 14(18):19195. https://doi.org/10.2807/ese.14.18.19195-en
102. Frisvad JC, Thrane U, Samson RA, Pitt JI (2006) Important mycotoxins and the fungi which produce them. In: Advances in food mycology. Springer, Boston, pp 3–31
103. Vidal JC, Bonel L, Ezquerra A, Hernández S, Bertolín JR, Cubel C, Castillo JR (2013) Electrochemical affinity biosensors for detection of mycotoxins: a review. Biosens Bioelectron 49:146–158. https://doi.org/10.1016/j.bios.2013.05.008
104. Prabhakar N, Matharu Z, Malhotra BD (2011) Polyaniline Langmuir-Blodgett film based aptasensor for ochratoxin A detection. Biosens Bioelectron 26(10):4006–4011. https://doi.org/10.1016/j.bios.2011.03.014
105. Castillo G, Lamberti I, Mosiello L, Hianik T (2012) Impedimetric DNA Aptasensor for sensitive detection of Ochratoxin A in food. Electroanalysis 24(3):512–520. https://doi.org/10.1002/elan.201100485
106. Evtugyn G, Porfireva A, Sitdikov R, Evtugyn V, Stoikov I, Antipin I, Hianik T (2013) Electrochemical APTASENSOR for the determination of Ochratoxin A at the Au electrode modified with Ag nanoparticles decorated with macrocyclic ligand. Electroanalysis 25 (8):1847–1854. https://doi.org/10.1002/elan.201300164
107. Hayat A, Sassolas A, Marty JL, Radi AE (2013) Highly sensitive ochratoxin A impedimetric aptasensor based on the immobilization of azido-aptamer onto electrografted binary film via click chemistry. Talanta 103:14–19. https://doi.org/10.1016/j.talanta.2012.09.048
108. Rivas L, Mayorga-Martinez CC, Quesada-González D, Zamora-Gálvez A, de la Escosura-Muñiz A, Merkoçi A (2015) Label-free impedimetric aptasensor for ochratoxin-A detection using iridium oxide nanoparticles. Anal Chem 87(10):5167–5172. https://doi.org/10.1021/acs.analchem.5b00890
109. Mejri-Omrani N, Miodek A, Zribi B, Marrakchi M, Hamdi M, Marty JL, Korri-Youssoufi H (2016) Direct detection of OTA by impedimetric aptasensor based on modified polypyrrole-dendrimers. Anal Chim Acta 920:37–46. https://doi.org/10.1016/j.aca.2016.03.038
110. Wei M, Zhang W (2017) A novel impedimetric aptasensor based on AuNPs–carboxylic porous carbon for the ultrasensitive detection of ochratoxin A. RSC Adv 7 (46):28655–28660. https://doi.org/10.1039/c7ra04209d
111. Simão EP, Cao-Milán R, Costa-Pedro G, De Melo CP, Cao R, Oliveira MDL, Andrade CAS (2017) Simple and fast picomolar detection of ochratoxin A using a reusable label free aptasensor built with a layer-by-layer procedure. Electroanalysis 29(10):2268–2275. https://doi.org/10.1002/elan.201700290
112. Castillo G, Spinella K, Poturnayová A, Šnejdárková M, Mosiello L, Hianik T (2015) Detection of aflatoxin B 1 by aptamer-based biosensor using PAMAM dendrimers as immobilization platform. Food Control 52:9–18. https://doi.org/10.1016/j.foodcont.2014.12.008

113. Li Q, Lu Z, Tan X, Xiao X, Wang P, Wu L, Shao K, Yin W, Han H (2017) Ultrasensitive detection of aflatoxin B1 by SERS aptasensor based on exonuclease-assisted recycling amplification. Biosens Bioelectron 97:59–64. https://doi.org/10.1016/j.bios.2017.05.031
114. Istamboulié G, Paniel N, Zara L, Reguillo Granados L, Barthelmebs L, Noguer T (2016) Development of an impedimetric aptasensor for the determination of aflatoxin M1 in milk. Talanta 146:464–469. https://doi.org/10.1016/j.talanta.2015.09.012
115. Chen X, Huang Y, Ma X, Jia F, Guo X, Wang Z (2015) Impedimetric aptamer-based determination of the mold toxin fumonisin B1. Microchim Acta 182(9–10):1709–1714. https://doi.org/10.1007/s00604-015-1492-x
116. Commission Regulation (EU) No 165/2010 (2010) Commission Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins
117. Karapetis S, Nikolelis D, Hianik T (2018) Label-free and redox markers-based electrochemical aptasensors for aflatoxin M1 detection. Sensors 18(12):4218. https://doi.org/10.3390/s18124218
118. Malir F, Ostry V, Pfohl-Leszkowicz A, Malir J, Toman J (2016) Ochratoxin A: 50 years of research. Toxins 8(7):191. https://doi.org/10.3390/toxins8070191
119. Amézqueta S, González-Peñas E, Murillo M, López de Cerain A (2004) Validation of a high-performance liquid chromatography analytical method for ochratoxin A quantification in cocoa beans. Food Addit Contam 21(11):1096–1106. https://doi.org/10.1080/02652030400019422
120. Yoo SM, Lee SY (2016) Optical biosensors for the detection of pathogenic microorganisms. Trends Biotechnol 34(1):7–25. https://doi.org/10.1016/j.tibtech.2015.09.012
121. Amiri M, Bezaatpour A, Jafari H, Boukherroub R, Szunerits S (2018) Electrochemical methodologies for the detection of pathogens. ACS Sensors 3(6):1069–1086. https://doi.org/10.1021/acssensors.8b00239
122. Arora P, Sindhu A, Dilbaghi N, Chaudhury A (2011) Biosensors as innovative tools for the detection of food borne pathogens. Biosens Bioelectron 28(1):1–12. https://doi.org/10.1016/j.bios.2011.06.002
123. Teng J, Yuan F, Ye Y, Zheng L, Yao L, Xue F, Chen W, Li B (2016) Aptamer-based technologies in foodborne pathogen detection. Front Microbiol 7:1426–1426. https://doi.org/10.3389/fmicb.2016.01426
124. Kant K, Shahbazi M-A, Dave VP, Ngo TA, Chidambara VA, Than LQ, Bang DD, Wolff A (2018) Microfluidic devices for sample preparation and rapid detection of foodborne pathogens. Biotechnol Adv 36(4):1003–1024. https://doi.org/10.1016/j.biotechadv.2018.03.002
125. Labib M, Zamay AS, Kolovskaya OS, Reshetneva IT, Zamay GS, Kibbee RJ, Sattar SA, Zamay TN, Berezovski MV (2012) Aptamer-based viability impedimetric sensor for bacteria. Anal Chem 84(21):8966–8969. https://doi.org/10.1021/ac302902s
126. Jia F, Duan N, Wu S, Dai R, Wang Z, Li X (2015) Impedimetric *Salmonella* aptasensor using a glassy carbon electrode modified with an electrodeposited composite consisting of reduced graphene oxide and carbon nanotubes. Microchim Acta 183(1):337–344. https://doi.org/10.1007/s00604-015-1649-7
127. Bagheryan Z, Raoof JB, Golabi M, Turner APF, Beni V (2016) Diazonium-based impedimetric aptasensor for the rapid label-free detection of *Salmonella typhimurium* in food sample. Biosens Bioelectron 80:566–573. https://doi.org/10.1016/j.bios.2016.02.024
128. Sheikhzadeh E, Chamsaz M, Turner APF, Jager EWH, Beni V (2016) Label-free impedimetric biosensor for *Salmonella typhimurium* detection based on poly [pyrrole-co-3-carboxyl-pyrrole] copolymer supported aptamer. Biosens Bioelectron 80:194–200. https://doi.org/10.1016/j.bios.2016.01.057
129. Ma X, Jiang Y, Jia F, Yu Y, Chen J, Wang Z (2014) An aptamer-based electrochemical biosensor for the detection of *Salmonella*. J Microbiol Methods 98:94–98. https://doi.org/10.1016/j.mimet.2014.01.003

130. Ranjbar S, Shahrokhian S, Nurmohammadi F (2018) Nanoporous gold as a suitable substrate for preparation of a new sensitive electrochemical aptasensor for detection of *Salmonella typhimurium*. Sensors Actuators B Chem 255:1536–1544. https://doi.org/10.1016/j.snb.2017.08.160
131. Salam F, Tothill IE (2009) Detection of *Salmonella typhimurium* using an electrochemical immunosensor. Biosens Bioelectron 24(8):2630–2636. https://doi.org/10.1016/j.bios.2009.01.025
132. Ozalp VC, Bayramoglu G, Kavruk M, Keskin BB, Oktem HA, Arica MY (2014) Pathogen detection by core-shell type aptamer-magnetic preconcentration coupled to real-time PCR. Anal Biochem 447:119–125. https://doi.org/10.1016/j.ab.2013.11.022
133. Labib M, Zamay AS, Kolovskaya OS, Reshetneva IT, Zamay GS, Kibbee RJ, Sattar SA, Zamay TN, Berezovski MV (2012) Aptamer-based impedimetric sensor for bacterial typing. Anal Chem 84(19):8114–8117. https://doi.org/10.1021/ac302217u
134. Dua P, Ren S, Lee SW, Kim JK, Shin HS, Jeong OC, Kim S, Lee DK (2016) Cell-SELEX based identification of an RNA aptamer for *Escherichia coli* and its use in various detection formats. Mol Cells 39(11):807–813. https://doi.org/10.14348/molcells.2016.0167
135. Burrs SL, Bhargava M, Sidhu R, Kiernan-Lewis J, Gomes C, Claussen JC, McLamore ES (2016) A paper based graphene-nanocauliflower hybrid composite for point of care biosensing. Biosens Bioelectron 85:479–487. https://doi.org/10.1016/j.bios.2016.05.037
136. Yao L, Wang L, Huang F, Cai G, Xi X, Lin J (2018) A microfluidic impedance biosensor based on immunomagnetic separation and urease catalysis for continuous-flow detection of *E. coli* O157:H7. Sensors Actuators B Chem 259:1013–1021. https://doi.org/10.1016/j.snb.2017.12.110
137. Vanegas DC, Rong Y, Schwalb N, Hills KD, Gomes C, McLamore ES (2015) Rapid detection of *listeria* spp. using an internalin A aptasensor based on carbon-metal nanohybrid structures. In: Proceedings of SPIE – smart biomedical and physiological sensor technology XIISPIE Sensing Technology + Applications. SPIE, Baltimore. https://doi.org/10.1117/12.2177441
138. Sidhu R, Rong Y, Vanegas DC, Claussen J, McLamore ES, Gomes C (2016) Impedance biosensor for the rapid detection of *listeria* spp. based on aptamer functionalized Pt-interdigitated microelectrodes array. In: Cullum BM, Kiehl D, McLamore ES (eds) Proceedings of SPIE – smart biomedical and physiological sensor technology XIIISPIE Commercial + Scientific Sensing and Imaging. SPIE, Baltimore. https://doi.org/10.1117/12.2223443
139. Jia F, Duan N, Wu S, Ma X, Xia Y, Wang Z, Wei X (2014) Impedimetric aptasensor for *Staphylococcus aureus* based on nanocomposite prepared from reduced graphene oxide and gold nanoparticles. Microchim Acta 181(9–10):967–974. https://doi.org/10.1007/s00604-014-1195-8
140. Reich P, Stoltenburg R, Strehlitz B, Frense D, Beckmann D (2017) Development of an impedimetric aptasensor for the detection of *Staphylococcus aureus*. Int J Mol Sci 18(11):2484. https://doi.org/10.3390/ijms18112484
141. Xiong X, Shi X, Liu Y, Lu L, You J (2018) An aptamer-based electrochemical biosensor for simple and sensitive detection of *staphylococcal* enterotoxin B in milk. Anal Methods 10(3):365–370. https://doi.org/10.1039/c7ay02452e
142. Commission Regulation (EC) No 2073/2005 (2005) Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs
143. Lee YJ, Han SR, Maeng J-S, Cho Y-J, Lee S-W (2012) In vitro selection of *Escherichia coli* O157:H7-specific RNA aptamer. Biochem Biophys Res Commun 417(1):414–420. https://doi.org/10.1016/j.bbrc.2011.11.130
144. Rubab M, Shahbaz HM, Olaimat AN, Oh D-H (2018) Biosensors for rapid and sensitive detection of *Staphylococcus aureus* in food. Biosens Bioelectron 105:49–57. https://doi.org/10.1016/j.bios.2018.01.023
145. Evenson ML, Ward Hinds M, Bernstein RS, Bergdoll MS (1988) Estimation of human dose of *staphylococcal* enterotoxin A from a large outbreak of *staphylococcal* food poisoning

involving chocolate milk. Int J Food Microbiol 7(4):311–316. https://doi.org/10.1016/0168-1605(88)90057-8
146. Benvidi A, Banaei M, Tezerjani MD, Molahosseini H, Jahanbani S (2017) Impedimetric PSA aptasensor based on the use of a glassy carbon electrode modified with titanium oxide nanoparticles and silk fibroin nanofibers. Mikrochim Acta 185(1):50. https://doi.org/10.1007/s00604-017-2589-1
147. Yang ZH, Zhuo Y, Yuan R, Chai YQ (2017) Amplified impedimetric aptasensor combining target-induced DNA hydrogel formation with pH-stimulated signal amplification for the heparanase assay. Nanoscale 9(7):2556–2562. https://doi.org/10.1039/c6nr08353f
148. He L, Zhang S, Ji H, Wang M, Peng D, Yan F, Fang S, Zhang H, Jia C, Zhang Z (2016) Protein-templated cobaltous phosphate nanocomposites for the highly sensitive and selective detection of platelet-derived growth factor-BB. Biosens Bioelectron 79:553–560. https://doi.org/10.1016/j.bios.2015.12.095
149. Jahanbani S, Benvidi A (2016) Comparison of two fabricated aptasensors based on modified carbon paste/oleic acid and magnetic bar carbon paste/Fe3O4@oleic acid nanoparticle electrodes for tetracycline detection. Biosens Bioelectron 85:553–562. https://doi.org/10.1016/j.bios.2016.05.052
150. Jarczewska M, Kékedy-Nagy L, Nielsen JS, Campos R, Kjems J, Malinowska E, Ferapontova EE (2015) Electroanalysis of pM-levels of urokinase plasminogen activator in serum by phosphorothioated RNA aptamer. Analyst 140(11):3794–3802. https://doi.org/10.1039/c4an02354d
151. Chen Z, Chen L, Ma H, Zhou T, Li X (2013) Aptamer biosensor for label-free impedance spectroscopy detection of potassium ion based on DNA G-quadruplex conformation. Biosens Bioelectron 48:108–112. https://doi.org/10.1016/j.bios.2013.04.007
152. Shi P, Zhang Y, Yu Z, Zhang S (2017) Label-free electrochemical detection of ATP based on amino-functionalized metal-organic framework. Sci Rep 7(1):6500. https://doi.org/10.1038/s41598-017-06858-w
153. Gopinath SC (2007) Methods developed for SELEX. Anal Bioanal Chem 387(1):171–182. https://doi.org/10.1007/s00216-006-0826-2
154. Stoltenburg R, Reinemann C, Strehlitz B (2007) SELEX--a (r)evolutionary method to generate high-affinity nucleic acid ligands. Biomol Eng 24(4):381–403. https://doi.org/10.1016/j.bioeng.2007.06.001
155. Wehbe M, Labib M, Muharemagic D, Zamay AS, Berezovski MV (2015) Switchable aptamers for biosensing and bioseparation of viruses (SwAps-V). Biosens Bioelectron 67:280–286. https://doi.org/10.1016/j.bios.2014.08.033
156. Radi AE, O'Sullivan CK (2006) Aptamer conformational switch as sensitive electrochemical biosensor for potassium ion recognition. Chem Commun 32:3432–3434. https://doi.org/10.1039/b606804a
157. Zhang Z, Yang W, Wang J, Yang C, Yang F, Yang X (2009) A sensitive impedimetric thrombin aptasensor based on polyamidoamine dendrimer. Talanta 78(4–5):1240–1245. https://doi.org/10.1016/j.talanta.2009.01.034
158. Su W, Lin M, Lee H, Cho M, Choe WS, Lee Y (2012) Determination of endotoxin through an aptamer-based impedance biosensor. Biosens Bioelectron 32(1):32–36. https://doi.org/10.1016/j.bios.2011.11.009
159. Xu H, Gorgy K, Gondran C, Le Goff A, Spinelli N, Lopez C, Defrancq E, Cosnier S (2013) Label-free impedimetric thrombin sensor based on poly(pyrrole-nitrilotriacetic acid)-aptamer film. Biosens Bioelectron 41:90–95. https://doi.org/10.1016/j.bios.2012.07.044
160. Tran DT, Vermeeren V, Grieten L, Wenmackers S, Wagner P, Pollet J, Janssen KP, Michiels L, Lammertyn J (2011) Nanocrystalline diamond impedimetric aptasensor for the label-free detection of human IgE. Biosens Bioelectron 26(6):2987–2993. https://doi.org/10.1016/j.bios.2010.11.053

161. Du Y, Li B, Wei H, Wang Y, Wang E (2008) Multifunctional label-free electrochemical biosensor based on an integrated aptamer. Anal Chem 80(13):5110–5117. https://doi.org/10.1021/ac800303c
162. Yurkovetsky ZR, Linkov FY, Malehorn DE, Lokshin AE (2006) Multiple biomarker panels for early detection of ovarian cancer. Future Oncol 2(6):733–741. https://doi.org/10.2217/14796694.2.6.733
163. Komarova E, Reber K, Aldissi M, Bogomolova A (2010) New multispecific array as a tool for electrochemical impedance spectroscopy-based biosensing. Biosens Bioelectron 25 (6):1389–1394. https://doi.org/10.1016/j.bios.2009.10.034
164. Lee J, Cho S, Lee J, Ryu H, Park J, Lim S, Oh B, Lee C, Huang W, Busnaina A, Lee H (2013) Wafer-scale nanowell array patterning based electrochemical impedimetric immunosensor. J Biotechnol 168(4):584–588. https://doi.org/10.1016/j.jbiotec.2013.08.029
165. Periyakaruppan A, Arumugam PU, Meyyappan M, Koehne JE (2011) Detection of ricin using a carbon nanofiber based biosensor. Biosens Bioelectron 28(1):428–433. https://doi.org/10.1016/j.bios.2011.07.061
166. Qureshi A, Gurbuz Y, Niazi JH (2010) Label-free detection of cardiac biomarker using aptamer based capacitive biosensor. Procedia Eng 5:828–830. https://doi.org/10.1016/j.proeng.2010.09.236
167. Lim T, Lee SY, Yang J, Hwang SY, Ahn Y (2016) Microfluidic biochips for simple impedimetric detection of thrombin based on label-free DNA aptamers. Biochip J 11 (2):109–115. https://doi.org/10.1007/s13206-016-1203-7
168. Lum J, Wang R, Hargis B, Tung S, Bottje W, Lu H, Li Y (2015) An impedance aptasensor with microfluidic chips for specific detection of H5N1 avian influenza virus. Sensors 15 (8):18565–18578. https://doi.org/10.3390/s150818565
169. Wang Y, Ye Z, Ping J, Jing S, Ying Y (2014) Development of an aptamer-based impedimetric bioassay using microfluidic system and magnetic separation for protein detection. Biosens Bioelectron 59:106–111. https://doi.org/10.1016/j.bios.2014.03.015
170. Zubtsov DA, Ivanov SM, Rubina AY, Dementieva EI, Chechetkin VR, Zasedatelev AS (2006) Effect of mixing on reaction–diffusion kinetics for protein hydrogel-based microchips. J Biotechnol 122(1):16–27. https://doi.org/10.1016/j.jbiotec.2005.08.010
171. Feng S, Chen C, Wang W, Que L (2018) An aptamer nanopore-enabled microsensor for detection of theophylline. Biosens Bioelectron 105:36–41. https://doi.org/10.1016/j.bios.2018.01.016
172. Liao W, Cui XT (2007) Reagentless aptamer based impedance biosensor for monitoring a neuro-inflammatory cytokine PDGF. Biosens Bioelectron 23(2):218–224. https://doi.org/10.1016/j.bios.2007.04.004
173. Keefe AD, Cload ST (2008) SELEX with modified nucleotides. Curr Opin Chem Biol 12 (4):448–456. https://doi.org/10.1016/j.cbpa.2008.06.028
174. The Eyetech Study Group (2003) Anti-vascular endothelial growth factor therapy for subfoveal choroidal neovascularization secondary to age-related macular degeneration: phase II study results. Ophthalmology 110(5):979–986. https://doi.org/10.1016/S0161-6420(03)00085-X
175. González-Fernández E, de-los-Santos-Álvarez N, Lobo-Castañón MJ, Miranda-Ordieres AJ, Tuñón-Blanco P (2011) Impedimetric aptasensor for tobramycin detection in human serum. Biosens Bioelectron 26(5):2354–2360. https://doi.org/10.1016/j.bios.2010.10.011
176. Rowe AA, Miller EA, Plaxco KW (2010) Reagentless measurement of aminoglycoside antibiotics in blood serum via an electrochemical, ribonucleic acid aptamer-based biosensor. Anal Chem 82(17):7090–7095. https://doi.org/10.1021/ac101491d