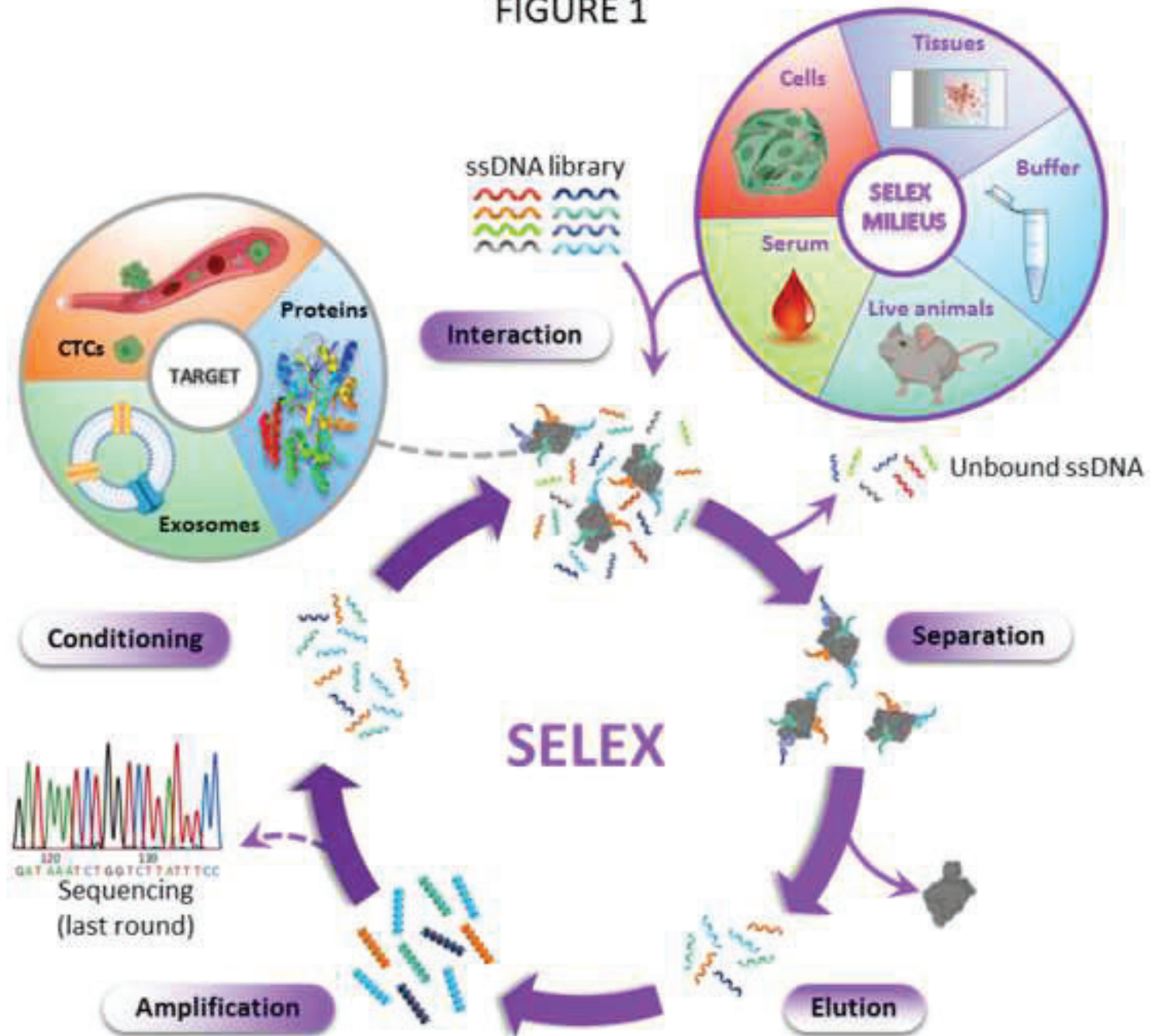


FIGURE 1



Electrochemical aptasensors for cancer diagnosis in biological fluids – A review

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Abstract

1
2 The tunability of SELEX procedure is an essential feature to supply bioaffinity
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4 receptors (aptamers) almost on demand for analytical and therapeutic purposes. This
5
6 longstanding ambition is, however, not straightforward. Non-invasive cancer diagnosis,
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8 so called liquid biopsy, requires collection of body fluids with minimal or no sample
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10 pretreatment. In those raw matrices, aptamers must recognize minute amounts of
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12 biomarkers that are not unique entities but large sets of variants evolving with the
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14 disease stage. The susceptibility of aptasensors to assay conditions has driven the
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16 selection of aptamers to natural environments to ensure their optimum performance in
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18 clinical samples. We present herein a compilation of the SELEX procedures in natural
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20 millieus. By revising the electrochemical aptasensors applied to clinical samples for
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22 cancer diagnosis and tracing back to the original SELEX we analyze whether aptamers
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24 raised using these SELEX strategies are being incorporated to the diagnostic devices
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26 and how aptasensors are finding their way to a market dominated by antibody-based
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28 assays.
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39 **Keywords:** aptamer; cancer; CTCs; electrochemical biosensors; exosome; SELEX,
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1. Introduction

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2 Early cancer diagnosis, before imaging revealing the tumor or even clinical symptoms,
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4 is an extremely active multidisciplinary research area. Fundamental pillars are the
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6 availability of proper biomarkers that excel in clinical sensitivity and specificity as well
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8 as proper analytical methodologies that make affordable large population screenings.
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10 Unfortunately, today the only biomarker in clinical usage for this task is the prostate
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12 specific antigen (PSA), which is strongly questioned because of the increasing number
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14 of unnecessary interventions in indolent or slow-growing tumors that boost the health
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16 public budget and the anxiety and discomfort of patients [1].
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20 All FDA-approved cancer biomarkers with prognostics, predictive and monitoring
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22 ability are detected by immunoassays, but the advent of novel receptors has triggered
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24 the development of methods with enhanced analytical features that enable improving
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26 the clinical performance of known biomarkers. That way, the selection of aptamers,
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28 since their discovery at the beginning of the 90's [2, 3], has increased notably, not only
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30 for diagnostic purposes but also as therapeutic agents or, more recently, for theranostic
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32 devices [4, 5].
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39 In the first two decades of this century, the accumulated knowledge at all biochemical
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41 levels has grown extraordinarily fueled by the (analytical) methodologies that made the
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43 deciphering of human genome a sooner reality than expected. Early cancer diagnosis
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45 and recurrence must benefit from it through the genome wide analysis of mutations, and
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47 the epigenetic and post-translational modifications (PTM) of nucleic acids and proteins.
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49 Novel biomarkers within these niches are currently under investigation with the
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51 expectation of finding the gold standard biomarker for each (sub)type of this diverse
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53 disease.
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1 The detection of epigenetic marks or PTM on proteins is an actual analytical challenge
2 because it involves a fraction of low abundant biomolecules. Alterations in these
3 features can change fundamental properties of each molecule that allow direct
4 electrochemical detection [6, 7], but receptor-based detection is more attractive because
5 it is target specific. These research avenues require the development of economic,
6 selective, renewable, and reproducible affinity receptors to unravel the function of
7 myriads of proteoforms [8] and its clinical effectiveness to guide cancer management.
8 Antibody reproducibility issues, openly recognized [9], are being battled [10, 11]. In
9 fact, there are programs such as the National Cancer Institute's Antibody
10 Characterization aimed at "*having three monoclonal antibodies produced for each*
11 *successfully expressed/purified recombinant antigen and one antibody per peptide (1 to*
12 *3 peptides per protein)*" [12], which must be extensively characterized under public and
13 rigorous guidelines. Aptamers are the receptors of choice when wishing to direct the
14 recognition to specific sites on a biomarker, typically a protein. The modular concept of
15 *in-vitro* selection of aptamers, schematized in Figure 1, is very suitable to include steps
16 to eliminate sequences that bind undesired regions of the biomolecule. For details on
17 SELEX procedure and its variants we refer the readers to specific and excellent reviews
18 [13, 14]. Batch to batch variation is not a concern with chemically synthesized
19 aptamers, but similar matters regarding the poor characterization are now evident [15-
20 17] and need to be addressed.

21 Since the first electrochemical aptasensor [18], a variety of aptamer-based
22 electrochemical devices to detect cancer biomarkers have appeared. Those detecting
23 protein tumor biomarkers or, more recently CTCs, have been partially reviewed within a
24 wider scope in terms of transduction [19-21], field of application [22, 23] or type of
25 bioreceptors [24, 25]. Other reviews are focused on a specific protein tumor biomarker

1 [26-28], type of targets [29] or certain aspects of aptamer-based approaches such as the
2 use of nanomaterials [30, 31]. Our approach covers for the first time the three targets
3 enabling liquid biopsy: protein biomarkers, CTCs, and exosomes. The latter has not
4 been reviewed except in a brief paragraph within a very recent compilation of optical
5 approaches [32] and a short perspective on DNA-based biosensors (genosensors and
6 aptasensors) [33].

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14 As long as the reported methods have not been tested in clinical samples their
15 usefulness is not fully demonstrated. This is why, in contrast to previous reviews, our
16 critical discussion is restricted to those. The main goal of the discussion is not the
17 detailed enumeration of every single assay scheme, but to emphasize the suitability for
18 its implementation in clinical settings. We trace back the aptamer sequence to the
19 original selection process to ascertain how the selection environment determines and
20 influences its subsequent application in body fluids. Thus, we first review the viability
21 of using biological media or samples during the SELEX process to secure the native
22 state of the target and ensure the successful functioning of aptamers in the *in-vitro*
23 diagnostic device. This is the most often claimed pitfall with respect to antibodies [34],
24 which are less sensitive to medium variations. The core of the revision is a critical
25 analysis of aptamers used in electrochemical biosensors for the detection of proteins,
26 circulating tumor cells (CTCs) and exosomes as consolidated or potential cancer
27 biomarkers. They represent an accurate picture of the whole aptasensors world in terms
28 of advantages and shortcomings and its potential to reach the market of medical
29 diagnostic devices, aspects commented in the last section of this review.
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54 **2. Aptamer selection in biological media**

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57 An early frontier, soon surpassed, in aptamer selection was the requirement of purified
58 single targets. Cell-SELEX first showed the suitability of complex matrices for the
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1 aptamer selection [35], with the benefit of preserving the native state of targets but at
2 the expense of not knowing what the ultimate target is unless costly and time-
3 consuming non-straightforward post-SELEX experiments are carried out (typically in
4 combination with mass spectrometry (MS)). At the same time, it becomes clear that
5 recombinant proteins synthesized through genetic engineering in prokaryotic systems,
6 easy to purify and widely used as targets, may generate aptamers that do not interact
7 with the corresponding native counterpart. This is attributed to PTM in eukaryotes that
8 do not take place in prokaryotic cells. In fact, native proteins are not unique molecules.
9 On the contrary, each one is a set of proteoforms arising from DNA and RNA-levels
10 variations, errors in transcription and translation, PTMs, exposure to toxic compounds
11 and response to pathological states [8].
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26 Therefore, selection of aptamers to unpurified proteins in biological media, such as
27 serum, tissues, and post-operative samples, has attracted great interest [36] (Figure 1).
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29 An important advantage of SELEX procedures in raw materials is that they do not
30 require deep biochemical knowledge to select the target in advance, but careful and
31 more intensive counter-selection steps must be introduced to eliminate non-specific
32 binding events. Besides, as a side-result, these strategies are indirect ways of biomarker
33 screening, after proper identification.
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43 Last year has witnessed the first attempts of using whole serum as an appealing
44 complex target to generate aptamers for diagnostic purposes [37-39]. In all cases pooled
45 serum from cancer diagnosed patients is used in positive rounds of selection. The
46 extraordinary problem of cancer heterogeneity, low abundancy of specific biomarkers
47 and discrimination from benign diseases is addressed by means of counter steps with
48 pooled serum from healthy individuals. That is, the selection is unselectively directed
49 toward any difference between both types of serum. DNA degradation by nucleases
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1 after repeated interactions in serum is not reported. Both heterogeneous and in-solution
2 SELEX are proposed. The heterogeneous strategy is based on the immobilization of the
3
4 serum components on carboxylated magnetic beads (MBs), enabling magnetic
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6 separation to discard non-specific bound DNA (negative selection) and unbound DNA
7
8 (positive selection) [37, 38]. Alternatively, the homogeneous approach makes use of
9
10 neutral polyacrylamide gel electrophoresis to separate bound and unbound fractions,
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12 instead of magnetic beads [39]. However, both strategies suffer from several
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14 shortcomings. On the one hand, immobilization of serum components seems a limiting
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16 strategy not only because the binding capacity of MBs but also because it restricts the
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18 targets to those containing functional groups that readily react with them. The lack of
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20 auxiliary reagents to activate the negatively-charged $-COOH$ groups, low reactive at
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22 physiological pH, imposes an additional not well understood restriction [37, 38]. Only
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24 in one case, depletion of major proteins is performed before indiscriminate
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26 immobilization of serum components on (MB) [38]. In fact, control experiments with an
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28 unrelated aptamer (selected against *E. coli.*) show significant binding to cancer serum
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30 components, raising questions about the tumor specificity of the aptamer and the
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32 identity of the target. On the other hand, the proposed homogeneous SELEX
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34 complicates the separation of bound fractions [39]. Although pioneering, this SELEX
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36 approach has certain weaknesses. Despite tumors from distinct organs share biomarkers,
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38 sera from patients with different types of cancer were not used in negative selection
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40 steps. Counterintuitively, the obtained aptamers using serum from gastric cancer
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42 showed selectivity against other cancers (lung, colorectal or hepatocellular) even though
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44 among the MS-assigned candidate targets are molecules present in several cancers [37].
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46 Some of the aptamers derived in serum show promising clinical performance in
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1 hepatocellular cancer, but more strict studies in patients with other types of cancer are
2 needed [39].
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4 In the above described SELEX, the abnormal components of the serum are the targets.
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6 Beyond this application, human serum can be used to mimic the real environment. In an
7 early attempt, simulated trypsinized human plasma (25%) was used to isolate aptamers
8 to be employed in MB-pull down prior to MS. When the aptamer was tested in human
9 serum samples, the binding buffer required supplementation with EDTA to chelate
10 divalent ions in order to allow the correct folding of the aptamer [40], showing an ion-
11 dependent functioning of the selected aptamer. This is a typical example of the need for
12 exact matching the selection milieu.
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15 Post-operative samples as a source of targets for aptamer selection are also of great
16 interest. The protein expression in tumor cell cultures might be different from that in
17 primary tumor tissues, thus aptamers generated through classic Cell-SELEX might not
18 achieve the analytical requirements to be applied in clinical specimens. With this idea in
19 mind, Zamay *et al.* developed aptamers to lung adenocarcinoma cells derived from post-
20 operative tissues, using lymphocytes and normal lung cells in the negative selections
21 [41]. The cancer variability is addressed because each round is performed with fresh
22 samples from different patients. The aptamers selected after 11 rounds bind to various
23 candidate proteins identified by HPLC-HR-MS based on probability scoring. In support
24 of this approach, these aptamers recognize the targets on CTCs and extracellular
25 vesicles in peripheral blood of lung cancer patients, while they only weakly bind to
26 cancer culture cells. The weak point is the need for a precise coordination between
27 surgeons and laboratory teams, effort which is not always at hand. One of the aptamers
28 was finally used for magnetic isolation of targets from crude blood plasma samples and
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1 for their subsequent electrochemical quantification, allowing to observe significant
2 differences between healthy and cancer patients [42].
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4 Similarly to post-operative samples, tissue slides have been employed to develop
5 aptamers [43-46]. A remarkable advantage of tissue sections is that they are more
6 representative of the cancer morphology than cultured cell lines, thus allowing the
7 evolution of aptamers against different histological sections, including the extracellular
8 matrix, the membrane components, and intracellular targets [43, 46]. Image-guided
9 selection of aptamers that bind to tumor vasculature or tumor cells but not to stromal
10 cells has been achieved using a laser beam to cut the region of interest [44]. Recently,
11 an automatic microfluidic system has reduced the selection time to 15 h [45]. A possible
12 drawback of tissue-SELEX is that the process of tissue slicing, immobilization, and
13 staining with chemical reagents might lead to the denaturation of the target proteins on
14 the tissue slide [36].
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31 Other alternative routes to obtain aptamers in unpurified biological media include *in*
32 *vivo* SELEX, which consists of the selection of aptamers able to specifically bind
33 tumors in living animals [47-51]. Such methods rely on the direct injection of the
34 nucleic acid library into the animal and the extraction of the target tissue after binding,
35 which abolishes one of the most attractive characteristics of these receptors: animal-free
36 selection. These approaches also require modified oligonucleotides to skip the nuclease
37 degradation and are mostly aimed at the development of aptamers for targeted-drug
38 delivery, and so are beyond the scope of this review.
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53 **3. Electrochemical aptasensors for protein tumor biomarkers**

54 In general, aptamer selection against tumor biomarkers is performed in “biological”
55 buffers, namely phosphate buffer saline and then tested in body fluids. Most of the
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1 electrochemical aptasensors have been tested in blood fractions because it is also the
2 most tested body fluid in hospitals and there is reliable information on the clinical
3 performance. Urine [52, 53] and recently saliva [54] are slowly appearing as alternative
4 samples, though they are fully non-invasive and easy to collect from healthy
5 individuals.
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11 There are still many cases where only artificially spiked samples were tested. Though it
12 is a step toward real applications, accessibility to clinical samples should be compulsory
13 to draw accurate conclusions. Currently, there are aptamers selected against FDA-
14 approved biomarkers such as alpha-fetoprotein (AFP) [55, 56], PSA [57-61],
15 carcinoembryonic antigen (CEA) [62-69], HER2/neu [70, 71] or carbohydrate antigen
16 125 (CA125) [72-74]; and potential cancer biomarkers such as mucin 1 (MUC1) [62],
17 neuron specific enolase (NSE) [67], osteopontin [75], vascular endothelial growth factor
18 (VEGF₁₆₅) [76, 77], platelet-derived growth factor (PDGF) [52, 53], epidermal growth
19 factor receptor (EGFR) [78] and interleukin-6 (IL-6) [54].
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33 Even aptamers against unknown targets are starting to be applied. This is the case of a
34 direct aptasensor for the detection of lung cancer-related proteins [42]. In this case, the
35 standards for calibration are purified proteins using streptavidin MB from blood of lung
36 cancer patients eluted in urea. This means that the aptamer is able to recognize the
37 denatured protein target. The aptamer whose sequence, including the primers, is 5'-CTC
38 CTC TGA CTG TAA CCA CGT GCC CGA ACG CGA GTT GAG TTC CGA GAG
39 CTC CGA CTT CTT GCA TAG GTA GTC CAG AAG CC-3' is stable in contact with
40 crude plasma samples up to 5 h due to the preincubation of the aptamer-modified Au
41 electrode with masking DNA [42].
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55 When the target is predefined, purified native or recombinant proteins are used during
56 the SELEX. In the case of MUC1, highly expressed by the majority of human
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1 adenocarcinomas and indicating a poor survival ratio, several types of recombinant
2 proteins were used as targets [79, 80], which yielded a set of aptamers. The one with the
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4 sequence 5'- GCA GTT GAT CCT TTG GAT ACC CTG G-3' was applied to detect
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6 the protein shed to the bloodstream [62] but also to isolate CTCs (see section 4).
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9 It has been argued that RNA aptamers are better suited for binding due to
10 conformational diversity but, for analytical applications, DNA aptamers are superior
11 and preferred because they are less prone to nuclease degradation. Importantly, direct
12 translation of an RNA sequence to obtain the DNA counterpart able to bind the same
13 target does not work [16, 34]. The absence of 2'-OH groups strongly affects the
14 recognition ability so the resultant aptamer is not functional. SELEX using DNA or
15 RNA libraries selects sequences completely different, though the enrichment process
16 depends on additional designing factors. As an example, Meirinho *et al.* used an RNA
17 aptamer previously described to bind human recombinant osteopontin (5'-CGG CCA
18 CAG AAU GAA AAA CCU CAU CGA UGU UGC AUA GUU G-3') [81] with
19 precautions to avoid degradation. Contrarily to the original aptamer, it was not
20 synthesized with 2'-OMe cytosine and uracyl nucleotides and therefore it was only
21 applied in buffer solutions [82]. The analytical application in serum samples required a
22 new selection from a DNA library against the recombinant human protein. The winning
23 sequence (5'-TGT GTG CGG CAC TCC AGT CTG TTA CGC CGC-3') does not
24 match the RNA one [75].
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48 Most aptasensors use the thiol-Au chemistry to anchor an aptamer on the transducer.
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50 The stability of these mixed self-assembled monolayers (SAMs) is shorter than a month
51 [57, 83] limiting the potential commercialization. Ternary SAMs have been reported to
52 triplicate stability at least in genosensors [84] but they have not been widely adopted.
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58 Paper-based aptamer-containing devices showed superior stability than mixed SAMs,
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though they also rely on AuNPs for immobilization [67]. Of note, 96.7% of signal remains after 4 weeks, higher percentage than using an antibody in an identical sensor layer, showing the improved stability of synthetic receptors.

Magnetic beads-based assays are appealing because of the compatibility with automation/microfluidics widely used in clinical labs. In one aptasensor for CEA using a 18-nt long aptamer (in italics) elongated to form a hairpin (5'-CCA *CGA TAC CAG CTT ATT CAA TTC* GTG G-3'), the streptavidin-modified MBs were used to entrap the biotinylated aptamer-CEA complex formed on Janus nanoparticles. The biotin tag was only accessible after recognition due to aptamer conformational switching [63]. However, the calibration by single standard additions complicates its clinical applicability. Another strategy is tethering the CEA aptamer to the MB by amide-coupling [66].

Biosensors require the receptor is in intimate contact with the transducer. An extended but not recommended trend includes under this definition homogeneous assays where the electrode is only used to measure the redox activity of a soluble probe. In a typical example of those, the transduction is achieved after a highly complicated recycling scheme (Figure 2A). It involves the digestion of a methylene blue-tagged DNA, which ultimately adsorbs on the graphene/GCE electrode and provides the analytical signal. The 18-nt CEA aptamer is designed as a hairpin structure that is opened only in the presence of the target [68].

The dominant assay format is the direct detection with a single aptamer. Depending on the interrogating technique the analytical signal can increase or decrease when the target concentration increases. Increasing analytical signals are preferred because they are less prone to false positive results when the sensor platform is degraded. When impedance is measured, protein binding to aptamer tethered to the transducer surface usually yields

1 “signal-on” methods (Figure 2B). The detection of VEGF₁₆₅ with the aptamer 5'-TTT
2 CCC GTC TTC CAG ACA AGA GTG CAG GG-3' chemisorbed on Au-based
3 nanostructures is an example [76, 77]. The former sensor was also used in “signal-off”
4 mode by measuring the current of an electroactive molecule that binds to the aptamer
5 being displaced after protein recognition (Figure 2B). Similarly, other “signal-off”
6 approaches add redox probes in solution to measure their accessibility to the sensor
7 surface that is usually impeded after protein recognition [55, 57, 75, 76]. To further
8 decrease the electroactive surface area, the addition as blockers of hydrophobic beads
9 specific for high molecular weight proteins was reported to enhance the sensitivity of a
10 direct assay for lung cancer-related proteins by two orders of magnitude [42].
11 Attachment of the redox probe to the sensor layer makes the addition of any external
12 reagents unnecessary [59, 67]. For point-of-care (POC) applications, reagentless
13 methods are desirable. To this aim the use of self-reporting strands is the ideal strategy.
14 This requires that the aptamer structural switching, upon binding, brings the distal-
15 appended redox label close to the electrode surface. Plaxco's group pioneers this sensor
16 architecture and applied it to PDGF detection, but in undiluted serum the detectability
17 resulted significantly reduced [85]. This strategy is not of general application because it
18 requires a large conformational change, which is not always experienced by the
19 aptamers selected under affinity pressure rather than proper folding for sensing.
20 Besides, the LOD, defined by the total number of self-reporting aptamers on the surface,
21 does not reach those of conventional ELISA. Likewise, it is not easily coupled to
22 amplification schemes [86]. Nonetheless, this strategy is valuable for real-time detection
23 of small molecules in body fluids [87], where weak aptamer-ligand binding makes this
24 interaction reversible.
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1 Sandwich approaches are scarce in spite of the outcome of SELEX process being a
2 collection of high-affinity binders. However, the candidates must bind different sites,
3 which require additional screening experiments unless two identical binding sites exist.
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5 Aptamer truncation to the minimal length with the highest affinity is a post-SELEX
6 time-consuming, and costly work carried out through trial and error experiments or
7 more recently guided by molecular dynamics [88, 89]. Nonetheless, it is vividly
8 recommended to minimize undesired hybridization events between a pair of receptors in
9 sandwich formats and for economic reasons of the final device. CEA aptamers, for
10 example, contain after truncation of the original library just 18-nt (the above cited) and
11 19 nt (5'- AGG GGG TGA AGG GAT ACC C-3'), half the commonest length [65].
12
13 Recently, PSA aptamer candidates for a sandwich approach were scrutinized for
14 hybridization ability to minimize unspecific signals [61] (Figure 3A). The case of
15 PDGF-BB, one of the isoforms of the PDGF implicated in the cell transformation
16 process and in tumor growth and progression, is special because it is a homodimer.
17 Curiously, the original SELEX was performed against a recombinant PDGF-AB to
18 obtain aptamers that bind to the A chain but all screened aptamers exhibited preferential
19 binding to the B chain in spite of a 60% homology between them [90]. Sandwich
20 approaches with the aptamer that way obtained, 5'-CAG GCT ACG GCA CGT AGA
21 GCA TCA CCA TGA TCC TG-3', are straightforward [52].
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23 The need for high affinity aptamers is demonstrated in a sandwich aptasensor for HER2
24 protein. Direct replacement of one or two anti-HER-2/neu antibodies with aptamers
25 rendered lower sensitivity due to lower in-solution aptamer affinity or availability of
26 proper binding sites, although the limit of detection (LOD) was not affected [70]. The
27 aptamer used is a trimeric version of the original 14nt-long aptamer 5'-GCA GCG GTG
28 TGG GG-3', selected against a purified HER-2 protein from gastric cancer cells [91].
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1 The monomeric aptamer was also employed in a peptide-aptamer sandwich assay [71].

2 It was extended with a toehold sequence that served as initiator of a self-assembled
3 hybridization chain reaction. This aptasensor was tested in HER2⁺ and HER2⁻ breast
4 cancer patients and also after surgery corroborating with the postoperative decrease in
5 the biomarker level recorded with an ELISA method.
6

7 Mixed antibody-aptamer assays bypass the hybridization issue, usually with aptamer
8 acting as the signaling receptor. This format extends the amplification schemes to those
9 based on molecular biology tools. Six-fold increase in signal and 500-fold lower LOD
10 for CEA, a biomarker for several cancers, were achieved using a DNA isothermal
11 amplification scheme, Rolling Circle Amplification (RCA), and the 18-nt aptamer
12 sequence [69]. Hybridization chain reaction (HCR) that consists of a supersandwich
13 extension through two assisting DNA strands, also improved the detection of CA-125, a
14 glycoprotein elevated in ovarian cancer. In this approach, the aptamer 5'- TAA TAC
15 GAC TCA CTA TAG GGA GAC AAG AAT AAA CGC TCA A-3' was used and the
16 amplification power of HCR was increased by a branching growth on AuNPs [72]
17 (Figure 3B). The way of selection of this sequence as well as of other aptamers for this
18 biomarker (e.g. 5'-TTA TCG TAC GAC AGT CAT CCT ACA C-3') used in
19 electrochemical approaches [73, 74] is not declared.
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22 More rarely, aptamers act as capture elements while antibodies are the detection
23 receptors. This scheme has been evaluated in a EGFR sensor. In this case a biotinylated
24 aptamer anchored to streptavidin-MB recognized EGFR, and the AuNPs covered with
25 the corresponding antibody served for recognition and as a reporter after dissolution of
26 Au in acid [78]. Interestingly, this is one of the few examples of an RNA aptamer with
27 an untruncated, unusually long sequence 5'-GGC GCU CCG ACC UUA GUC UCU
28 GUG CCG CUA UAA UGC ACG GAU UUA AUC GCC GUA GAA AAG CAU
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1 GUC AAA GCC GGA ACC GUG UAG CAC AGC AGA-3'. Modification of the
2 nucleobases routinely needed to preclude nuclease degradation was not used.

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4 Interestingly, one-week storage stability and capability to discriminate between serum
5 from healthy and cancer patients is reported.
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8 The ingenious amplification schemes affordable only with aptamers have reached
9 noticeable LOD but increase the cost when enzymes (exonuclease strand recycling [68]
10 or isothermal DNA amplification schemes [69]) or auxiliary strands (hybridization-
11 based displacement between aptamer and complementary DNA [62, 73], HCR [71, 72]
12 or combination of both [66]) are needed. Likewise, the level of complexity increases
13 requiring trained personnel to obtain reproducible results.
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17 Multiplexing is a desirable characteristic of biosensors because there is increasing
18 evidence pointing out that early detection of cancer needs panels of biomarkers to be
19 simultaneously detected [92]. Dual aptasensors are emerging but only a few have been
20 tested in clinical samples [62, 67].
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24 Reusability, on the contrary, is not very common in clinical practice. Low-cost
25 disposable biosensors are commonly claimed as advantageous but when thousands of
26 analyses per month are performed the cost could become unaffordable. Reusable
27 biosensors are not easy to obtain without damaging the sensor layer. An aptasensor for
28 PSA claims easy regeneration by immersion in water at 70 °C for 1 h [57]. This
29 temperature is high enough to disassemble the aptamer-target complex while
30 maintaining the aptamer chemisorption on Au nanoparticles. The LOD of this aptasensor
31 is well below the decision threshold for prostate cancer diagnosis. Taking into account
32 that all healthy men have PSA content around this threshold, it is curious that serum from
33 healthy people gave signals similar to blank samples (in buffer). One possible
34 explanation is the use of serum from women but it was not specified. High temperatures
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1 are expected to disrupt the Au-thiol bond. Nonetheless, temperatures as high as 95 °C
2 for 5 min did not show evidence of degradation of the sensor biolayer after 5 cycles of
3 using a very long aptamer (5'-GTG ACG CTC CTA ACG CTG ACT CAG GTG CAG
4 TTC TCG ACT CGG TCT TGA TGT GGG TCC TGT CCG TCC GAA CCA ATC-3')
5 for AFP detection, a tumor biomarker in use at diagnosis of hepatocellular carcinoma
6 for disease staging [55].
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10 An innovative approach for the detection of protein biomarkers through both the peptide
11 and glycan regions has been first explored for PSA. The sandwich format uses the
12 Savory's *et al.* protein binding aptamer 5'-AAT TAA AGC TCG CCA TCA AAT
13 AGC-3' [93] to capture the protein and a glycan aptamer specifically derived for human
14 PSA (5'-GGA CGG TTG CGC TAT ATT TAA CCA AAA GTC TGG ATT AAC A-
15 3') [61] to recognize the glycosylated region of the protein (Figure 3A). Both aptamers
16 were selected in artificial buffers after exposure to the purified protein but the second
17 one was deliberately directed towards the sugar moiety. Interestingly, when deployed in
18 serum samples of patients with suspected prostate cancer, the aptasensor shows some
19 discordant result with respect to the reference electrochemiluminescence method used in
20 the hospital. Those punctual samples correspond to people with PSA content in the
21 "gray zone" (inconclusive range because elevated PSA content could be ascribed to
22 benign prostate conditions) or higher, who were finally diagnosed of something else.
23 The aptasensor was able to correctly classify them pointing to superior discrimination.
24 Nevertheless, the aptasensor should be tested in a larger cohort of serum samples to
25 draw robust conclusions.
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53 The main analytical features of the selected electrochemical approaches are summarized
54 in Table 1. Of note, the analytical figures of merit are mostly obtained in buffer except
55 in a few cases where the dynamic range and LOD is also explored in the body fluid.
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1 This information is essential in order not to overestimate the analytical performance in
2 clinical settings and for validation. The effects on the analytical features vary depending
3 on the type of sample, the transduction technique and the sensor architecture offering
4 different antifouling degrees. The graphene-based FET demonstrated good resistance to
5 fouling with similar performance in artificial gargle solutions and human saliva [54]. It
6 is quite interesting to compare the effect of serum on two similar immobilization
7 strategies: tethering the capture biotinylated aptamer to streptavidin covalently linked to
8 a carboxylic SAM. One of the approaches, the sandwich aptasensor for PSA showed
9 identical LOD in both media. The concentration range narrowed in serum due to the
10 hook effect (signal decrease at high concentrations) that is shifted to smaller
11 concentrations than in buffer [61]. In contrast, the osteopontin aptasensor showed worse
12 LOD by CV, but slightly lower by SWV. However, the regression equations lost
13 linearity ($r < 0.95$ vs 0.9995 in buffer) [75]. The difference might be the length of the
14 carboxylated alkanethiol chain; 3 carbons for the osteopontin sensor versus 11 for the
15 PSA one, and the lack of passivating thiol in the former. Finally, an interesting case is
16 detection of using Janus nanoparticles and MBs. It shows a three-fold loss in sensitivity
17 that worsens the LOD, although the sample is not in contact with the transducer, which
18 is considered favorable to minimize unspecific signals, and a high dilution of the serum
19 is carried out (1:100). The assay still covers the clinical range [63].

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46 In general, the number of samples tested are rather limited (from 1 [63, 75, 77] to 21
47 [59]). Validation with other routine methods is not always presented and when it is
48 estimated as trueness (bias), it ranges between 2% [58] and 18 [76]– 23% [57].

4. Electrochemical aptasensors for CTCs

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Circulating tumor cells (CTCs) are cells detached from primary tumors that enter the bloodstream or the lymphatic system, where they invade distant sites of the body and form metastatic tumors, eventually giving rise to cancer-associated deaths [94]. They are considered as “treasure chests” because harbor proteins and nucleic acids that provide unique information about the tumor. Therefore, the role of CTCs as minimally invasive cancer biomarkers would be twofold. Their concentration is meaningful for cancer prognosis and treatment monitoring; while their cargo carries valuable information to facilitate the implementation of personalized therapy.

Despite their promising applications in cancer management, the rare occurrence of CTCs in blood (1-100 CTCs among billions of healthy blood cells in 1 mL of whole blood is clinically significant) along with the limited blood sample volume available (~5-10 mL) hampers their sensitive and specific detection. In consequence, prior to CTC analysis, they are usually captured and preconcentrated. There is a plethora of methods for CTC isolation [95] that could be roughly divided into two large groups. The first one comprises traditional methods based on physical properties of tumor cells such as size, density, electrical charge and deformability. Although intact CTCs are recovered in a cost-effective way, they suffer from limited purity as a result of their low selective isolation. The second group encompasses those methods based on biological features, namely on the affinity binding of specific antigens present on the surface of CTCs. To this group belongs CellSearch®, the only commercial product approved by the US Food and Drug Administration for CTC detection. It relies on immunomagnetic capture of CTCs of epithelial origin by using an anti-EpCAM antibody attached to magnetic particles. However, some CTCs might escape isolation due to dynamic changes in tumor cells such as epithelial-mesenchymal transition.

1 Being functional nucleic acids obtained by chemical synthesis, aptamers possess
2 intrinsic features that make them a better choice for specific separation and release of
3 CTCs. Particularly interesting are their small size, high thermal and chemical stability,
4 and easy modification with functional groups for efficient isolation of CTCs. Likewise,
5 the release of captured CTCs while keeping cells viability could be carried out by
6 reversible thermal disruption of affinity binding or nuclease cleavage, among other
7 strategies [96]. Table 2 describes the distinctive features of the aptamer-based assays for
8 CTCs detection in body fluids. Of note, all analytical characteristics were obtained in
9 buffer.

10 The aptamer most commonly used for the design of electrochemical aptamer-based
11 cytoassays is an aptamer against EpCAM, a transmembrane glycoprotein exclusively
12 expressed in epithelial cells and epithelial derived neoplasm. This is a 48-nt DNA
13 sequence so-called SYL3C (5'-CAC TAC AGA GGT TGC GTC TGT CCC ACG TTG
14 TCA TGG GGG GTT GGC CTG-3'), obtained by a SELEX process which uses the
15 purified recombinant protein as the target [97], but it has been proven to recognize
16 EpCAM both on cell surfaces and exosomes. Direct recognition of breast cancer cell
17 line MCF-7 is possible [98] as well as in combination with antibody [99] or a glycan-
18 binding protein [100], which indicates different binding sites for aptamer and antibody
19 on the protein moiety and lack of affinity for the sugar region.

20 CTCs usually carry more than one of the target proteins, which make sandwich
21 approaches more frequently designed than in the case of free protein detection. The
22 25nt-long MUC1 aptamer above mentioned acted as capturing and signaling receptor to
23 detect MCF-7 cell line in spiked serum [101] and blood [102].

24 Aptamers for CTC detection are not limited to those raised against purified proteins.
25 When searching for discriminating features between normal and tumor cells, Cell-

1 SELEX-derived aptamers are of great utility. Aptamers specific of leukemia cells
2 (CCRF-CEM) were obtained by using a Burkitt's lymphoma cell line (Ramos cells) as
3
4 negative control [103]. Optimization of the best binders based on their secondary
5
6 structure led to a 41-nt long aptamer named sgc8c (5'-ATC TAA CTG CTG CGC CGC
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8 CGG GAA AAT ACT GTA CGG TTA GA-3') [104], widely used in aptasensing.
9
10 Specifically, electrochemical aptassays were applied to CCRF-CEM added to serum
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12 samples [105-107] or clinical samples from patients [108]. The target of sgc8c aptamer
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14 turned out to be PTK7-5, an isoform of protein tyrosine kinase 7 [109]. Selective
15
16 recognition of Burkitt's lymphoma cells can be also achieved with a 44-nt aptamer
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18 TD05 (5'-ACC GGG AGG ATA GTT CGG TGG CTG TTC AGG GTC TCC TCC
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20 CGG TG-3') with immunoglobulin heavy Mu chain (IGMN) as binding site [35].
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22 Despite omitting the counter selection step in Cell-SELEX process, cytoassays based on
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24 TD05 aptamer showed specific binding to Ramos cells in the presence of breast (MCF-7
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26 and SK-BR-3), lung (A549) and leukemia (CCRF-CEM) cancer cell lines [108].
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34 A panel of aptamers has been selected to detect CTCs derived from liver cancer, among
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36 which TLS1c (5'-ACA GGA GTG ATG GTT GTT ATC TGG CCT CAG AGG TTC
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38 TCG GGT GTG GTC ACT CCT G-3') and TLS11a (5'-ACA GCA TCC CCA TGT
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40 GAA CAA TCG CAT TGT GAT TGT TAC GGT TTC CGC CTC ATG GAC GTG
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42 CTG-3') stand out for their high affinity. They have been used separately [110] or
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44 combined [111].
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49 The G-quadruplex forming DNA aptamer (5'-GGT GGT GGT GGT TGT GGT GGT
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51 GGT GG-3') known as AS1411 targeting nucleolin protein has been used in
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53 electrochemical sensors directed to different cancer cells: breast (MCF-7 and MB231)
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55 lung (A549), and cervical (HeLa) [102, 112]. This aptamer has advanced up to clinical
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1 trial phase II for acute myeloid leukemia treatment but Antisoma, the proprietary
2 company, terminated it due to inconclusive results.
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4 Chemisorption on Au surfaces is the dominant surface chemistry to attach the aptamer
5 to the transducer. To maximize the accessibility of capture aptamers and thus, the target
6 binding capability, DNA nanotetrahedron (NTH) structures are used as efficient
7 bioscaffolds [102, 110]. Alternatively, the binding site of the aptamer can be extended
8 away from the surface with an extra 19-nt long fragment for partial hybridization of the
9 aptamer with a surface-tethered complementary probe [98]; this approach favors the
10 reutilization of the aptasensor while circumventing surface steric hindrance. This is also
11 achieved by elongation of the aptamer sequence at the proximal end to carbon surfaces,
12 e.g. with 15 extra thymines [111]. Carbodiimide-mediated attachment of amine-
13 functionalized aptamers to carbon surfaces with carboxylic acid groups is also a simple
14 strategy [111, 112].
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31 To increase the number of receptors anchored to the electrode surface, and thus the
32 device sensitivity, as well as speeding up the electron transfer between the redox
33 molecule and the electrode, conducting nanomaterials such as porous graphene oxide
34 [112] or reduce graphene oxide decorated with gold nanoparticles [101] are extremely
35 useful.
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43 Furthermore, capturing CTCs with aptamer-modified MB [99, 100] or magnetic
44 nanocarriers [108] and subsequent detection onto an electronic surface can provide
45 superior analytical performances because both steps are carried out under their own
46 optimal conditions. These approaches cannot be considered as genuine aptasensors
47 because the biorecognition event does not take place on the transducer [113].
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56 Regarding the assay format, direct aptassays involve the molecular recognition of the
57 target cancer cells using the cognate aptamer immobilized onto the sensing interface
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1 (Figure 4). Using impedance as a non-destructive non-passivating detection technique,
2 the signal-on approach to quantify EpCAM⁺ tumor cells enabled the efficient recovery
3 of culturable cancer cells by enzymatic cleavage of several uracil bases in the aptamer
4 sequence [98] (Figure 4A). That way, the cells can be employed in downstream
5 applications and the electrodes are reusable.
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11 Among “signal-off” approaches, the decrease in the oxidation current of ascorbic acid
12 was employed to detect leukemia cells, boosted about 20 times upon localized surface
13 plasmon resonance excitation [105]. This amplification strategy led to a LOD of 5 cells
14 mL⁻¹ as well as a wide linear response range, although tedious pretreatment of blood
15 samples is required. Another interesting “signal-off” approach involves a light
16 addressable potentiometric sensor (LAPS) based on a Si₃N₄-SiO₂-Si n type structure
17 modified with porous graphene oxide for increasing the active sensing area [112].
18
19 Despite a modest detection limit, it provides results in good agreement with cancer
20 staging. Direct recognition of CTCs was also carried out with the aptamer receptor
21 immobilized onto an array nanochannel-ion channel hybrid by recording the drop-off in
22 the ionic flow through the nanochannels. Although its clinical application remains to be
23 demonstrated, release of viable CTCs was achieved by endonuclease digestion, thus
24 promoting subsequent genomic and proteomic studies [107] (Figure 4B).
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43 Compared to the previous single-receptor approaches, sandwich assays usually provide
44 enhanced selectivity due to the participation of a pair of receptors (capture and reporter
45 ones). Likewise, they favor a great variety of amplification strategies, which can be
46 classified into two large groups: (i) those based on the oligonucleotide nature of
47 synthetic receptors and (ii) those based on the unique advantages of nanoparticles
48 (Figure 5).
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1 Beyond DNA amplification strategies relying on the extension of the reporter aptamer
2 with a proper primer thus triggering isothermal and linear RCA [99, 110], nucleic acid
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4 nanomachines such as DNA walkers have finally found analytical interest to improve
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6 aptassay sensitivity [106] (Figure 5A). However, their sophisticated design involving
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8 DNA-modifying enzymes along with strand displacement reactions hampers its general
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10 applicability in clinical settings by the moment.
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14 Nanoparticles are versatile tools for signal amplification. As nanocarriers, nanoparticles
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16 can load multiple units of electroactive species (ferrocene or thionine) [108] (Figure
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18 5B), or HRP-mimicking DNAzymes [102] (Figure 5C) per recognition event that, in
19
20 combination with sandwich electrochemical aptassays, gave rise to good analytical
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22 performance, although it is true that the presence of natural HRP is still needed. There is
23
24 also a clear trend to replace natural redox enzymes by catalytic nanomaterials as
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26 reporter molecules. In this regard, CuO [101] (Figure 5D) and Pd@Au nanoparticles
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28 [100] exhibit hydrogen peroxide reduction activity. Beyond an improved stability
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30 against temperature and pH changes, their peroxidase activity is lower than that of the
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32 natural counterpart.
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36 Antifouling strategies such as a previous blocking step with a solution containing
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38 healthy blood cells have been reported when interrogating blood samples [111] even
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40 though two types of aptamers were used to detect a single type of CTCs.
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48 **5. Electrochemical aptasensors for exosomes**

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51 Cancer-derived exosomes constitute another attractive target for potential diagnostics,
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53 prognostics and surveillance of the disease. Exosomes are a kind of extracellular
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55 vesicles with a size in the range 30-150 nm in diameter, which transmit molecular
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57 messengers between cells, thus contributing to the sustained growth, invasion and
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1 metastasis of cancer cells [114]. They have their origin in the fusion of internal vesicles
2 with the plasma membrane to be released from parent cells to the extracellular fluid,
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4 moving to adjacent areas or tissues. Consequently, they are present in different body
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6 fluids including blood, urine, cerebrospinal fluid, saliva or breast milk, where they carry
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8 diverse molecules that are characteristic of the parent cell. Although exosomes have
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10 been discovered in the 80s, a recent flurry of work has shown that they hold great
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12 potential in cancer liquid biopsy [115].
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16 This new class of circulating cancer biomarkers is more abundant than CTCs, and is
17
18 relatively stable. The exosomal cargos, such as oncoproteins, RNA and DNA fragments,
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20 provide information about the primary tumor and its environment in a minimally
21
22 invasive way. In addition, as cancer cells secrete more exosomes than their non-
23
24 malignant counterparts, the selective counting of cancer-specific exosomes offers
25
26 valuable information for diagnosis. Despite the potential advantages of using exosomes
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28 as biomarkers listed above, their nanometric size poses important technical challenges
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30 for clinical translation. The majority of existing technologies for exosome detection and
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32 quantification require a previous isolation and enrichment step, which is typically
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34 accomplished by ultracentrifugation, ultrafiltration, polymer precipitation or
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36 immunoaffinity purification [116]. Nanoparticle tracking analysis (NTA) is the most
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38 popular technique for obtaining information about the size and concentration of
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40 exosomes after their isolation [117]. This is a light-scattering technique, only based on a
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42 physical property of exosomes, and in consequence it cannot support differentiation of
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44 phenotypes and subpopulations of the extracellular vesicles.
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52 To improve the selectivity of detection, it is necessary to select exosome-specific
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54 surface markers which would direct the selective capture and detection. Among the
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56 components of membrane exosomes there are clusters of tetraspanins, a family of
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1 membrane proteins also referred as the transmembrane 4 superfamily proteins, which
2 include CD9, CD63 or CD81, together with adhesion molecules such as integrins or the
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4 epithelial cell adhesion molecule (EpCAM) [114]. These surface proteins are typically
5
6 caught by specific antibodies to selectively capture and detect the exosomes.
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9 Although the use of aptamers for the integration of capture and recognition of the
10
11 extracellular vesicles is less than 5 year-old, examples of electrochemical aptamer-
12
13 based-sensors and assays for exosomes are compelling and growing.
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16 The most widely employed aptamer so far for the design of electrochemical aptamer-
17
18 based assays for exosomes is an aptamer against CD63 [118-125], a member of
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20 tetraspanin family and a type III lysosomal membrane protein that is a classic marker
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22 for exosomes. This is a 32-nt aptamer developed by Base Pair Biotechnologies [126],
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24 with the sequence: 5'-CAC CCC ACC TCG CTC CCG TGA CAC TAA TGC TA-3'. A
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26 truncated version of this aptamer, with just 10nt from the 5'-terminus, has proven
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28 effective for CD63 recognition [124]. As CD63 is presented in different types of
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30 exosomes, the corresponding aptamer has been employed for the capture or detection of
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32 exosomes secreted by different cancer cells such as breast (MCF-7) [118-122] and liver
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34 (HepG2) [123-125] cell lines. No information is available about the target used in the
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36 selection process or the affinity of it. Another ubiquitously expressed cancer-biomarker
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38 is EpCAM, whose 48-bases long aptamer mentioned above is also suitable for binding
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40 to exosomes released by, for example, breast [118, 119] or prostate [127] cancer cells.
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42 The MUC1 aptamer 5'-TAC TGC ATG CAC ACC ACT TCA ACT A-3', selected in
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44 buffer against a recombinant form different from the protein employed for selecting the
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46 aptamer used in serum and CTCs [80], has served for the selective detection of
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48 exosomes from gastric cancer cells in combination with antibodies anti-CD63 for
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50 capturing [128].
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It is possible to improve the selectivity of the detection by selecting as a target a cancer-specific marker on the surface of exosomes. This is the case of prostate specific membrane antigen (PSMA), which is an exopeptidase overexpressed on the apical plasma membrane of prostate cancer cells. The DNA aptamer with the sequence: 5'-GCG TTT TCG CTT TTG CGT TTT GGG TCA TCT GCT TAC GAT AGC AAT GCT-3' has been explored for the direct detection of exosomes associated to prostate cancer [127] or indirectly by measuring the release of an auxiliary cDNA that hybridized on the electrode [129].

To preclude binding to normal cell features, Cell-SELEX is a useful source of aptamers for exosomes. This was the approach followed to select an aptamer against tumor liver cells, LZH8: 5'-ATC CAG AGT GAC GCA GCA TAT TAG TAC GGC TTA ACC CPC ATG GTG GAC ACG GTG GCT TAG T. In addition, two artificial nucleotides Z and P were introduced in the initial library in order to improve the probability of selecting the best binder. The nucleobases form three hydrogen bonds and do not disturb the Watson-Crick double helix so they are compatible with polymerases [130]. Considering the homology between the membrane proteins of cells and secreted exosomes, this aptamer also exhibits selectivity for exosomes secreted by hepatocarcinoma cells (HepG2), discriminating them from those released by noncancerous liver cells (Hu1545), and in consequence it is an efficient receptor for the quantification of exosomes [131].

The development of aptamer-based electrochemical sensors requires attaching the aptamer to the transducer, and then measure of an electrical property directly or indirectly related to changes occurring when the exosomes are captured. To date, exosome aptasensors have been mainly fabricated by using gold surfaces, where aptamers are chemisorbed through thiol groups [121, 125, 127, 131] or using NH₂-

1 modified aptamers covalently linked to surfaces bearing carboxyl groups [118, 122].

2 The complex composition of human fluids and the low abundance of exosomes in these
3 samples, make the direct integration of selective recognition and detection not an easy
4 task. A way to overcome this problem is to apply modified MB for exosome separation
5 and enrichment, coupled to the downstream detection of the binding event. These have
6 been accomplished by immobilizing onto MB not only aptamers (CD63-aptamer [119]
7 or PSMA aptamer [129]) but also specific antibodies, for example antibodies
8 recognizing CD63 protein [123] or other proteins specifically binding molecules on the
9 surface of exosomes such as phosphatidylserine binding-protein (Tim 4) [124]. While
10 these detection schemes cannot be strictly considered aptasensors, it is possible to
11 integrate both steps in a microfluidic platform to analyze in a rapid and simple way
12 exosomes in clinical specimens for cancer diagnosis [124].

13 Electrochemical assays for exosome detection also fall into two classes i.e. direct and
14 sandwich assays. The first relies on the capture of exosomes by a single immobilized
15 specific aptamer. The simplest “signal-off” approach uses the electron-transfer
16 suppression of a soluble ferro/ferricyanide probe [131] (Figure 6A). In this device, an
17 oriented immobilization of the aptamer on Au and smart spacing, through a tetrahedral
18 DNA nanostructure, improves the accessibility of exosomes to the recognition element
19 and in consequence the sensitivity of the assay, reporting an LOD 100-fold lower than
20 the obtained with a classical SAM for the immobilization of the aptamer. Despite the
21 impressive sensitivity without any amplification strategy, the sensor was not challenged
22 in real samples, where probably the complexity of the matrix would lead to unspecific
23 adsorption and signal suppression (false positive). Displacement assays involve the
24 immobilization of the aptamer in the form of a duplex, hybridized with one or more
25 antisense DNA strands (Figure 6B) [125, 129]. The recognition of exosomes causes the

1 release of the complementary strands that are detected. It is very important to design the
2 hybridizing strands to be complementary to the aptamer in the region that is directly
3 involved in the recognition of the target. When the displaced strand is labelled with an
4 electroactive molecule, for example methylene blue, the recognition event produces a
5 decrease in the voltammetric signal of the marker, which is related to the exosome
6 concentration (secreted by liver cancer cells in the example) [125]. Another strand
7 displacement approach for the detection of exosomes from prostate cancer cells needed
8 3 cDNA strands, so the design is even more challenging [129]. The recognition event
9 takes place on the surface of MBs whereas the released DNA is detected by an
10 exonuclease III-assisted target recycling scheme on a separate gold electrode. A
11 decrease in the voltammetric signal ascribed to the soluble redox reporter, $\text{Ru}(\text{NH}_3)_6^{3+}$,
12 leads to a LOD surprisingly higher than the simplest design above mentioned [131],
13 despite the double-amplification process. The direct recognition of exosomes can also
14 be transformed into a DNA detection assay by derivatizing the exosomes, once
15 entrapped on the sensing layer, with a ssDNA sequence by a click chemistry process,
16 for example, where azide-labelled oligonucleotides are linked to alkynyl-4-one-
17 modified exosomes [121]. The ssDNA is then detected by HCR, using a biotin-hairpin
18 as a probe (Figure 6C). The final detection of the elongated DNA products is achieved
19 by electrochemically measuring the peroxidase activity of the streptavidin-peroxidase
20 conjugate used as a reporter. This is a very complicated scheme, which demands a long
21 derivatization process after exosome capturing, and although it has proven useful for
22 discriminating one breast cancer patient from one healthy individual, the design suffers
23 from a relatively poor LOD when compared with simpler assays.

24 The strategies described above make use of a heterogeneous reaction between
25 immobilized aptamers and exosomes, but the recognition reaction may also take place

1 in solution (homogeneous assay). The capture aptamer is hybridized with an
2 oligonucleotide, which is released upon exosome binding. These strands are detected on
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4 an ITO electrode, using doxorubicin as an electroactive probe, by an exonuclease III-
5 assisted recycling assay [120]. The design has the advantage of improving the binding
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7 efficiency of the exosomes, thus leading to improved detectability, although it has been
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9 only tested in spiked serum samples.
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12 Selectivity of the above designs is based exclusively on the selectivity of a single
13 aptamer used as a recognition element. To overcome this problem, sandwich-type
14 assays using a pair of aptamers as a capture and reporting receptor [118, 119, 122, 127]
15 or alternatively, an antibody [123, 128] or a surface lipid binding-protein [124] as a
16 capture element in combination with a reporting aptamer, have been developed (Figure
17 7). Different signal generation and amplification strategies have been employed in these
18 assays, such as electrooxidation of metal nanoparticles linked to the reporting aptamer
19 [127] (Figure 7A), catalytic effect of Ti_3C_2 MXenes nanosheets over
20 electrochemiluminescence of luminol [118], peroxidase activity of the complex between
21 hemin and a DNAzyme that elongates the aptamer [122, 124] (Figure 7C) and different
22 nucleic acid-based amplification strategies such as exonuclease III-assisted recycling
23 [119], cascade toehold-mediated strand displacement [123], and RCA [128] (Figure
24 7B). Table 3 summarizes the analytical performance of the published electrochemical
25 aptamer-based assays for exosome detection. All analytical features were obtained in
26 buffer, typically PBS, due to the need for isolation prior to detection. Integrated
27 collection and detection has not been reported using aptamers as a capture element. An
28 antibody-based electrochemical device has been designed to entrap salivary exosomes
29 and disrupted them by an electrical field to release their cargo [132]. The nucleic acid
30 content is then detected through hybridization with appropriate complementary probes
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1 anchored on a different electrode surface present in the chip. It is reasonable to envision
2 similar approaches with aptamers instead of antibodies in the future.
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4 Despite the inherent complexity associated with the use of nucleic acid amplification
5 strategies for signal generation, these designs do not provide much better sensitivity. Up
6 to date, the lowest LOD (9.5×10^2 particles mL^{-1}) is achieved in combination with RCA,
7 using an aptamer against MUC1 elongated with a primer sequence that is
8 complementary to a circular padlock containing a G-quadruplex unit [128]. After
9 recognition, RCA incorporates multiple G-quadruplex units that in the presence of
10 hemin show peroxidase-like activity, leading to a large amplification of the
11 voltammetric signal. However, the achieved LOD is very close to that obtained with
12 copper and silver nanoparticles as labels: 50 particles per sensor, which corresponds to
13 2×10^3 particles mL^{-1} for a volume of sample of 25 μL [127]. Both designs supported the
14 detection of exosomes in serum samples, and proved to be efficient in the
15 discrimination of healthy individuals from prostate cancer patients ($n=10$) [127] or
16 gastric cancer patients ($n=16$) [128]. Other designs also tested in the analysis of serum
17 samples from cancer patients, although in a fairly limited number, are linked to the use
18 of a DNAzyme sequence as a label, and were evaluated in the diagnosis of breast cancer
19 (8 patients) [122], and hepatic carcinoma (10 patients) [124].
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46 **6. The long path from lab bench to the clinical market**

47 The previous sections evidence the significant advancements that researchers have made
48 in the development of electrochemical aptasensors for cancer diagnosis. However, these
49 devices have not yet fulfilled their potential to be translated to the clinical market, so
50 that they can directly benefit patients. Challenges to take this great leap forward include:
51 a) to identify more specific tumor targets and b) to rational design the SELEX protocols
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1 to obtain robust well-characterized aptamers for clinically significant biomarkers, c) to
2 evaluate the clinical validity and utility of the new aptasensors, rather than simply their
3 analytical performance, and d) to develop simplest and integrated platforms useful in
4 POC systems.
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9 a. More specific tumor targets for the selection of aptamers.

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11 Cancer management requires collecting information about the disease stage and
12 evolution. These data cannot be gathered from repeated biopsies. The search for specific
13 biomarkers in the body fluids is emerging as a powerful tool, but their use as clinical
14 markers needs for selective receptors with an extraordinary ability to discriminate
15 minute changes within a defined target. Cancer is associated with a variety of aberrant
16 features whose detection might be more clinically significant. The AFP-L3 test based on
17 the ratio between the core-fucosylated protein and the whole protein is a successful
18 example [133]. Specific lectins are used to detect the aberrant fucosylation ratio.
19 Aptamers can replace them to recognize these or other subtle differences because they
20 can reach superior affinity. A first example to demonstrate the high clinical selectivity
21 that aptasensors might achieve in this direction is the discrepancies in PSA values
22 obtained with the aptasandwich assay recognizing both the protein and the glycan
23 moieties of PSA, and the reference method, just focused in the protein [61].
24 Nonetheless, this task will require exquisite accuracy because it is based on percentage
25 changes for the aberrant form of the protein with respect to its total amount.
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48 Future research should focus on novel biomarkers with enhanced sensitivity/selectivity
49 rather than on well-known biomarkers. Test currently in use will not be replaced unless
50 a substantial benefit from a clinical or economical point of view is apparent [134]. With
51 this aim, close collaboration between experts in the biomedical and analytical fields is
52 highly important. Interestingly, the use of affinity reagents different from the widely
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accepted antibodies has not demonstrated superior clinical specificity and sensitivity of the detection of the approved biomarkers. Protein-binding aptamers behave similar to antibodies in terms of affinity and selectivity, though the selection process of the latter can be tuned to boost both features. When the aptamer selection is targeting proteins on cell surface, the SELEX protocol should include several counter selections steps with numerous types of cells. Those essential steps to avoid cross-reactivity in the real application are rarely exploited. As a consequence, promiscuous aptamers binding several types of tumor cells through shared surface features are sometimes obtained [135]. Nonetheless, aptamers show benefits in reagent stability and in lowering the LOD through amplification schemes not amenable to antibodies. Extreme detectability might seem useless once the normal physiological level is reached, but it can lead to redefinition of the disease and clinical management [134].

Combination of several already described and novel biomarkers can unblock the unmet goal of early diagnosis of cancer and avoid confusion with biomarkers whose level also elevates with aging without implying enhanced cancer risk. This goal may be facilitated by the design of multiplexed aptasensors.

b. More aptamers in which we can trust

Another important question is the wide diversity in composition and properties of biological fluids, which should be considered in the design of the SELEX process. The proper aptamer recognition event is strongly dependent on the whole system: the aptamer sequence, the chemical nature of the target and the composition of the interrogating medium [34]. The best performance would require matching the selection conditions with the application to keep both the aptamer and target in their optimum chemical and structural conformations. The detailed inspection of aptamers used in clinical samples for cancer diagnostics reveals that the milieu in which they were

1 derived is not as decisive as it is commonly accepted. When targeting free proteins,
2 phosphate buffer saline sometimes supplemented with ions reasonably emulates the
3 body fluids so that the aptamers can easily recognize their target in clinical samples.
4 Good agreement with validated methods using different detection techniques further
5 support this finding, though more extensive studies are needed before entering the
6 commercial pipeline. It is worth noting that some of the aptamers mentioned above are
7 among the oldest developed and they have been tested repeatedly in several labs using a
8 variety of transduction techniques beyond electrochemistry. An exhaustive
9 characterization of aptamers is central to secure adequate performance and diminish the
10 risk of failure. Traceability of the aptamer back to the original selection is also
11 important to trust in the good performance.
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26 When the intended final use is to isolate/quantify CTCs or exosomes the selection of
27 aptamers in crude media is more often to preserve the native target state with its
28 inherent (aberrant) variants although the targets remain unidentified. This might
29 facilitate the detection of rare subtypes of tumor cells that skip current identification.
30 There is a clear need of expanding the collection of available aptamers against cancer-
31 specific biomarkers on the surface of CTCs, for reliable discrimination between them
32 and healthy blood cells. Furthermore, the use of multiple aptamers as ligands of several
33 biomarkers would promote the efficient capture of CTC subpopulations. The design of
34 multivalent capture devices is also a convenient option. AuNPs decorated with multiple
35 units of the sgc8 aptamer [136] or long DNA “tentacles” comprising repeated aptamer
36 sequences generated by RCA [137] have already demonstrated their high cell capture
37 efficiency in comparison with single units.
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55 c. Evaluation of the clinical validity and utility.
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When a laboratory assay faces an *in-vitro* medical test in specific clinical contexts, the excellence in analytical performance is taken for granted but clinical validity and utility must be demonstrated. Clinical validity (or performance) is the ability of the method to correctly classify patients with or without the disease or in a particular stage of it, while the clinical utility (or effectiveness) covers the health outcomes improvements. This includes patients' benefits and harms, as well as process outcomes, such as speeding up the time to diagnosis [134].

Within the economic evaluation, the cost per assay has an impact. Paper-based aptasensors claim a cost per assay below \$1 [67], but broader impacts are also considered. For example, for POC devices, implementation of biosensors can dramatically change the health organization system from large facilities to local units, which might be decisive to adopt the technology.

Integrated portable devices with wireless transmission of information to smartphones or cloud servers are useful to monitor the biomarker concentration trends and open the possibility to remote control by medical staff. Though a continuous monitoring of cancer biomarkers is not usually needed, this proposal can find applicability in order to reduce the inconvenience of attending doctor's office frequently. Recently, this type of device was reported for a cancer biomarker measured in saliva [54]. Companies like ApolloDx are developing electrochemical aptamer-based portable devices promising to detect cancer biomarkers though, up to date, they are only validated against infectious diseases [138].

d. Development of simple and integrated platforms for POC systems.

In POC simplicity is a must. Highly sensitive multistep protocols with amplification schemes, as those described, must demonstrate their reliability to be handled by untrained staff. One desirable feature of POC is the direct measurement in unpretreated

1 samples: for example, whole blood drops in glucometers. None of the methods reported
2 so far fulfill this need in blood. Most of them are applied to serum, meaning that
3 pretreatment is needed. Even crude plasma samples required centrifugation and
4 incubation steps [42]. Free protein detection in undiluted samples has been
5 accomplished in a few methods [59, 62, 67, 73], while the rest requires from twice to
6 ten-fold dilution, sometimes 100-fold or higher to reach the working range or eliminate
7 matrix effects from unspecific adsorption.
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9 CTCs and exosomes detection require specific isolation making this step crucial for
10 their accurate measurement. Integrated platforms based on microfluidics to isolate and
11 detect these targets are emerging. Aptamer-based devices such as Nano Velcro have the
12 ability to easily release the cells for downstream genetic and molecular profiling with a
13 cost for CTC enumeration about \$50, in contrast to \$1200 estimated with CellSearch[®]
14 [139]. Heterogeneity of exosomes makes isolation a current challenge that would
15 benefit from microfluidics and the future availability of sets of aptamers to confront the
16 cancer evolution under different physiological stages of the disease.
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18 If patent activity serves to forecast the position of a technology in the marketplace, the
19 potential of aptamer field is strong. A general search on Patentscope [140], using
20 aptamer as input, retrieves thousands of files. Beyond the initial patents on SELEX
21 protocol and its variants, the main interest in filing a patent on aptamers is their
22 therapeutic use. Nonetheless, diagnostic methods are also patented. Patent searching is a
23 daunting task because of the plurality of names under which “aptamer” is hidden.
24 Besides, the claims are extremely broad in order to encompass all variations of the
25 method patented, which might include different kinds of capture elements [141-143] or
26 a wide variety of targets [144] in the same patent file. Occasionally, the aptamer-based
27 methods to detect cancer biomarkers do not include a specific transduction technique
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1 but any diagnostic kit that uses the specific sequence claimed [145]. Among the
2 electrochemical techniques, impedance [146] or amperometry [147] stands out.
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4 ApolloDx have patented an electrochemical device based on the latter for wide
5 diagnostic testing [147]. Interestingly, at least two patents claim devices to detect cancer
6 biomarkers in saliva [142, 148]. The above mentioned patents focused on protein
7 targets. Recent patents claim the use of aptamers to capture extracellular vesicles from
8 tissues [149] or all body fluids, provided that the individual is suspected to have any
9 type of cancer [150]. Similarly, a microfluidic chip is claimed to capture CTCs by
10 means of aptamers for cancer-screening purposes. The same patent proposes the
11 eradication of malignant cells by injecting a photosensitizer-aptamer conjugate into the
12 bloodstream and application of a laser beam that destroy them [151]. Roche Diagnostics
13 Gmbh has recently filed a patent for a device to capture and analyze of CTCs. Besides
14 of antibodies, one of the embodiments comprises the use of aptamers [152].
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34 **7. Concluding remarks**

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36 There is no doubt that the electrochemical aptasensor field has increased enormously
37 and is now tackling the difficulties of shifting to the market. When using rigorously
38 characterized aptamers, their properties along with continuously improved selection
39 strategies indicate that there is no reason to expect delays in commercialization due to
40 technical issues of the receptor. Nonetheless, fighting the successful and long history of
41 antibodies, which are much more numerous than aptamers, is hard. Likewise, the
42 ignorance of the aptamers and their capabilities among investors and clinicians cannot
43 be set aside as an influencing factor that blocks the commercialization. We would like
44 to emphasize that the aim is not to substitute or withdraw antibodies. On the contrary,
45 the ultimate goal is to use aptamers in those applications where they outperform other
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affinity reagents and take advantages of their unique characteristics, such as animal-free selection. We believe that potential successful niches for aptamers might be targets that are inherently difficult targets for antibodies: low immunogenic compounds such as glycans, buried regions hard to access for large molecules or specific sites on molecules, e.g. aberrant modifications.

Electrochemical transduction should make easy the translation from the lab prototype to the marketable device. Antifouling strategies to protect against unspecific signals are extensively studied on a variety of electrode surfaces, but enhanced stability of the sensing layer is a weak point that needs to be addressed. As other biosensors relying on antibodies or other transduction techniques, the ultimate bottleneck in the commercial pipeline is providing evidences of clinical utility, in terms of both cost-effectiveness and patient and health system outcomes to convince stakeholders to invest in this young technology.

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Table 1: Summary of analytical characteristics of published electrochemical aptamer-based assays for free protein detection

Receptor	Sensing platform / Assay format	Detection method	Linear Range / LOD	Sample (number)	Ref.
AFP-aptamer	Spindle-shaped Au nanostructures on AuE / direct (signal off)	DPV of ferri/ferrocyanide	0.005-100 ng mL ⁻¹ / 0.23pg mL ⁻¹	S (20)	[55]
AFP apt	AuE / direct	DPV of ferri/ferrocyanide	0.01-100 pg mL ⁻¹ / 3.1 fg mL ⁻¹	S (3)	[56]
PSA apt	Au nanospers+AuE / direct (signal off)	DPV of ferri/ferrocyanide	0.125-200 ng mL ⁻¹ / 50 pg mL ⁻¹	S (10)	[57]
PSA apt	rG /AuNPs/ MWCNT /GCE/ direct	DPV of ferri/ferrocyanide (signal off) EIS	0.005–20 ng mL ⁻¹ /1pg mL ⁻¹ 0.005–100 ng mL ⁻¹ /1pg mL ⁻¹	S (4)	[58]
PSA apt	AuNPs/rGO/THI/ SPCE (signal off)	DPV of THI	0.05-200 ng mL ⁻¹ / 10 pg mL ⁻¹	S (21)	[59]
PSA apt	round hairbrush-like Au nanostructure / direct	DPV of methylene blue	0.125-128ng mL ⁻¹ / 50 pg mL ⁻¹	S (10)	[60]
Capt: PSA apt Sign:PSA-1 apt	Strep on AuE / sandwich	Chronoamperometry of TMB	0.66-62.5 ng mL ⁻¹ ** in buffer 0.66-25 ng mL ⁻¹ ** in serum/ 0.66 ng mL ⁻¹ (both)	S (15)	[61]
CEA apt hybridized to cDNA	AuE / strand displacement assay	DPV of metal ion labels + Ru(NH ₃) ₆ ³⁺	0.01 pM-100 nM/ 3.33 fM	S (5)	[62]
CEA apt	Au-SiO ₂ Janus NPs-CE / direct	Amperometry (enzyme amplification)	0.2-5000 ng mL ⁻¹ / 70 pg mL ⁻¹ in buffer 1-5000 ng mL ⁻¹ / 210 pg mL ⁻¹ in reconstituted serum	S (1)	[63]
CEA apt	rG+ Strep-CE/ direct	DPV of 2,3-diaminophenazine	5 pg mL ⁻¹ -50 ng mL ⁻¹ / 1.5 pg mL ⁻¹	S (8)	[64]
Capt: CEA apt 18-nt Sign: CEA apt 19-nt	hemin-GO-GCE / sandwich	Electrochemiluminescence	0.8-4 ng mL ⁻¹ / 0.28 pg mL ⁻¹	S (9)	[65]
CEA apt	MB-aptamer-cDNA (capture) NTH-cDNA2 on AuSPE (detection) Strand displacement	HCR+ DPV (hemin-G-quadruplex)	0.0001-50 ng mL ⁻¹ / 18.2 fg mL ⁻¹	S (5)	[66]
CEA apt	NG/THI/PB-AuNPs/ GCE on paper/ direct (signal off)	DPV of THI	0.01-500 ng mL ⁻¹ / 2 pg mL ⁻¹	S (15)	[67]
CEA apt	rGO on p-nitrophenyl modified GCE / homogeneous	DPV of adsorbed MB-ssDNA after exonuclease recycling	80 ag mL ⁻¹ -950 fg mL ⁻¹ / 80 ag mL ⁻¹	S (5)	[68]
Capt: anti-CEA Ab	GO-chitosan GCE / sandwich	RCA amplification + SWV of	0.5 pg mL ⁻¹ -1 ng mL ⁻¹ /	S (7)	[69]

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Sign: CEA apt		phosphomolybdate	0.1 pg mL ⁻¹		
Capt & sign: trimeric HER2-neu apt or Ab	Strep-MB / sandwich with cellulose labels	Chronocoulometry (nitrocellulose enzymatic degradation)	10 ⁻¹⁵ -10 ⁻¹⁰ / 1 fM***	S***	[70]
Capt: peptide Sign: monomeric HER2 apt	AuE / sandwich	HCR amplification + SWV of phosphomolybdate	1-100 pg mL ⁻¹ / 0.047 pg mL ⁻¹	S (6)	[71]
Capt: CA125 Ab Sign: CA125 apt	GO-chitosan GCE / sandwich	HCR on AuNPs + SWV of phosphomolybdate	100 μU mL ⁻¹ -10 U mL ⁻¹ / 50 μU mL ⁻¹	S (8)	[72]
CA125 apt	AgNPs amidoxime-modified nanofibers on ITO/ strand displacement	DPV of Methylene blue	0.01-350 U mL ⁻¹ / 0.0042 U mL ⁻¹	S (5)	[73]
CA125 apt	rGO and MWCNT /direct (signal off)	Field effect transistor	10 ⁻⁹ U mL ⁻¹ -1 U mL ⁻¹ / 5×10 ⁻¹⁰ U mL ⁻¹	S (1)	[74]
MUC1 apt hybridized to cDNA	AuE / displacement assay	DPV of metal ion labels and Ru(NH ₃) ₆ ³⁺ wires	0.01 pM-100 nM / 3.33 fM	S (5)	[62]
NSE apt	graphene/THI/PB-AuNPs-CE on paper/ direct (signal off)	DPV of PB	0.05-500 ng mL ⁻¹ / 10 pg mL ⁻¹	S (15)	[67]
Osteopontin apt	Strep on AuSPE / direct (signal off)	CV and SWV of ferri/ferrocyanide	CV: 25-100 nM / 2.6 nM _{in} buffer SWV: 12-100 nM / 1.4 nM _{in} buffer; 1.3 nM in serum	S (1)	[75]
VEGF ₁₆₅ apt	BSA-Au nanoclusters on IL-GCE / direct (signal off)	EIS (ferri/ferrocyanide) signal on DPV (methylene blue)	2.5-250 pM / 0.48 pM 1-120 pM / 0.32 pM	S (3)	[76]
VEGF ₁₆₅ apt	OMC-Au composite-SPE / direct	EIS (ferri/ferrocyanide)	10-300 pg mL ⁻¹ / 1 pg mL ⁻¹	S (1)	[77]
Capt & sign: PDGF-BB apt	AuNPs-rG-apt / sandwich	AuNP carrying apt and GOD + luminol ECL	0.1pM-0.5 nM / 17.4 fM	U (3)	[52]
PDGF-BB apt	VS ₂ /AuNP-GCE / direct (signal off)	DPV of ferri/ferrocyanide	1 pM -1 nM / 0.4 pM	U (5)	[53]
Capt: EGFR apt Sign: EGFR pAb	Strept-MB with apt (capt) + AuNPs-Ab (detection)	DPV of Au after acid dissolution	1-40 ng mL ⁻¹ * / 50 pg mL ⁻¹	S (9)	[78]
IL-6 apt	Graphene / direct (signal off)	Field effect transistor	0.05-20 nM** / 10.5 pM _{in} PBS 11.8 pM in artificial gargle 12.2 pM in human saliva	SL (3)	[54]
Apt for unknown lung cancer proteins	AuSPE / direct (signal off)	SWV of ferri/ferrocyanide	0.023-230 ng mL ⁻¹ / 0.023 ng mL ⁻¹	P (13)	[42]

*Two linear ranges **Non-linear calibration *** only tested with antibodies

Apt: aptamer; AuE: gold electrode; BSA: bovine serum albumin; Cap: capture; cDNA: complementary DNA; CE: carbon electrode; DPV: differential pulse voltammetry, ECL: electrochemiluminescence; EIS: electrochemical impedance spectroscopy; GCE: glassy carbon electrode; GOD: glucose oxidase, IL: ionic liquid; MWCNT: multi-walled carbon nanotubes; NTH: nanotetraedron; OMC: ordered mesoporous carbon; P: plasma; PB: prussian blue; rG: reduced graphene; S: serum; Sign: signaling; SL: saliva; SPE: screen printed electrode; SWV: square wave voltammetry; Strep: streptavidin; THI: thionine; U:urine

Table 2: Summary of analytical characteristics of published electrochemical aptamer-based assays for CTCs detection

Receptor	Sensing platform / Assay format	Detection method	Linear Range & LOD (cells mL ⁻¹)	Sample (number)	Ref.
EpCAM apt	AuE-cDNA partially hybridized with apt / Direct	EIS	$30 \times 10^6 / 10$	MCF-7 Spiked serum	[98]
Cap: EpCAM Ab Sign: EpCAM apt	MB (capt) + AuE electrode (detection) / Sandwich	RCA + SWV of phosphomolybdate	5-30000 / 1	MCF-7 (breast cancer) Spiked whole blood	[99]
Cap: EpCAM apt Sign: Concavalin A	Fe ₃ O ₄ @SiO ₂ -NH ₂ -NPs (capture) + AuE (detection) Sandwich	Pd@AuNPs catalysis Chronoamperometry	100-1×10 ⁶ / 30	MCF-7	[100]
Cap: MUC1 apt Sign: MUC1 apt	GCE-rGO-AuNPs Sandwich	DPV (CuO nanozyme)	50-7×10 ³ / 27	MCF-7 Spiked artificial human serum	[101]
AS1411 & MUC1 apt (both for Cap and Sign)	Au-NTH-bearing aptamers Sandwich	MOF-PtNPs decorated with apt and DNazyme- DPV	20-1×10 ⁷ / 6	MCF-7 Spiked in blood	[102]
PTK7 apt	GCE-Au nanostars Direct (signal off)	DPEE with AA	5-1×10 ⁵ / 5	CCRF-CEM Spiked in 10% human serum	[105]
Cap: PTK7 apt	AuNP-apt (capture) AuE-hairpin probe / strand displacement (signal off)	NEase-TCEP SWV of methylene blue	5-5000 / 1	CCRF-CEM Spiked in blood samples	[106]
Cap: PTK7 apt	Array nanochannel-ion channel hybrid (silanized PAA membrane-NH ₂ -aptamer) /direct	LSV (blockage of ionic flow through channels)	100-1000 / 100	CCRF-CEM	[107]
Cap & Sign of Ramos: Ig heavy Mu chain apt	Magnetic nanocomposite (capt) + CE (detection)	SWV Fc and THI attached to AuNPs	5-500 (both) / 4 (Ramos) and 3	Ramos & CCRF-CEM Spiked whole blood	[108]

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Cap & Sign of CCRF-CEM: PTK7 apt	Sandwich		(CCRF-CEM)	Blood from leukemia patients (3)	
Hepatocellular carcinoma-specific apt	Au-NTH +aptamer-PtNPs-primer RCA / Strand displacement	DPV of HRP-mimicking DNAzyme generated by RCA	$10^{-1} \times 10^6 / 3$	HepG2 Spiked in blood	[110]
Cap: two types of MEAR apt	GCE / Direct (signal off)	DPV of ferri/ferrocyanide	1-14 / 1	MEAR Spiked in blood	[111]
AS1411 apt	Si ₃ N ₄ -APTES-GO-CO ₂ H Direct (signal off)	LAPS	$5 \times 10^3 - 5 \times 10^6 / 5 \times 10^3$	HeLa, A549, MB231 Spiked in blood Blood breast cancer patients (8)	[112]

AA: ascorbic acid; DPEE: direct plasmon-enhanced electrochemistry; Fc: Ferrocene; HRP: horseradish peroxidase; LAPS: Light addressable potentiometric sensor; LSV: linear sweep voltammetry; MOF: metal organic framework; NEase: nicking endonuclease; PAA: porous anodic alumina; (r)GO: (reduced) graphene oxide; TCEP: Triggered cascade displacement polymerization;

Table 3: Summary of analytical characteristics of published electrochemical aptamer-based assays for exosome detection

Receptor	Sensing platform / Assay format	Detection method	Linear Range / LOD (part. mL ⁻¹)	Sample (number)	Ref.
Cap: EpCAM apt Sign: CD63 apt	AuNPs/GCE Sandwich	ECL of luminol catalyze by Ti ₃ C ₂ MXenes	$5 \times 10^5 - 5 \times 10^9$ 1.25×10^4	MCF-7 Spiked serum	[118]
Cap: CD63 apt Sign: EpCAM apt	MB (capt) + Au electrode (detection) Sandwich	DNAzyme-exonuclease activity- DNA release DPV of MB and Fc (ratiometric)	$5 \times 10^4 - 1 \times 10^{10}$ 1.2×10^4	MCF-7 Serum from breast cancer patients (3)	[119]
CD63 apt	Homogeneous, Direct ITO electrode	DNA release- ExoIII assisted recycling DPV Doxorubicin	$3.4 \times 10^4 - 3.4 \times 10^8$ 1.2×10^4	MCF-7 Spiked serum	[120]
CD63 apt	Au-rGO-GCE Direct- labelled exosomes	DNA linked to exosomes Hybridization chain reaction Streptavidin-HRP- DPV	$1.2 \times 10^5 - 1.2 \times 10^{11}$ 9.6×10^4	MCF-7 Serum from breast cancer patients (1)	[121]
Cap: CD63 apt Sign: CD63 apt- DNAzyme	GCE-CdS:Eu nanocrystals Sandwich (Signal-off)	DNAzyme/hemin ECL	$3.4 \times 10^5 - 1.7 \times 10^8$ 7.4×10^4	MCF-7 Serum from breast cancer patients (8)	[122]
Cap: CD63 Ab Sign: CD63 apt	MB (capture) + AuE (detection) Sandwich	Cascade toehold mediated strand displacement- MB duplex, SWV	$1 \times 10^5 - 5 \times 10^7$ 1.7×10^4	HepG2 Spiked serum	[123]

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Cap: Tim4 Sign: CD63 apt	MB (capture) + ITO (detection) Sandwich	DNAzyme-aptamer Hemin for peroxidase activity DPV of thionine	$7.6 \times 10^4 - 7.6 \times 10^8$ 4.4×10^3	HepG2 Serum from liver cancer patients (10)	[124]
CD63 apt	AuE Direct- displacement of MB- DNA (signal-off)	DPV of MB	$1 \times 10^6 - 1 \times 10^8$ 1×10^6	HepG2 Spiked serum	[125]
Cap: EpCAM apt Sign1: EpCAM apt Sign2: PSMA apt	Nanostructured AuE Sandwich	CuNP and AgNP electrooxidation- LSV	Not provided 2×10^3	VCap Serum from prostate cancer patients (10)	[127]
Cap: CD63 Ab Sign: MUC1 apt	AuE / Sandwich	RCA - hemin/DNAzyme reporter DPV	$4.8 \times 10^3 - 4.8 \times 10^6$ 9.5×10^2	SGC7901 Serum from gastric cancer patients (16)	[128]
PSMA apt	MB (capture) Direct, displacement DNA ($\times 3$)- AuE (signal off)	Exo III-assisted target recycling. DPV Ru(NH ₃) ₆ ³⁺ reporter	$1 \times 10^3 - 1.2 \times 10^5$ 7×10^4	LNCap Spiked serum	[129]
LZH8 apt	AuE / Direct (signal off)	SWV Ferro/ferricyanide	$1 \times 10^5 - 1 \times 10^{12}$ 2.1×10^4	HepG2	[131]

Tim4: Phosphatidylserine binding-protein

Captions for figures

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4 Figure 1: Scheme of the SELEX procedure including the common iterative steps:
5 interaction between the library and the target for cancer diagnosis (left wheel) that takes
6 place in one of the milieu indicated in the right wheel; separation of bound from
7 unbound sequences, elution of the bound sequences from the target to obtain a pool of
8 strands with higher affinity, PCR amplification of enriched pool and conditioning to
9 obtain single stranded DNA to start a new round of selection. Counter and negative
10 steps are not included for clarity. When the enrichment is sufficient, the last step is the
11 massive sequencing or alternatively cloning and sequencing of a limited number of
12 strands.
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23 Figure 2: Direct aptamer assays for the detection of protein tumor biomarkers. (A)
24 Homogeneous “signal-on” assay based on two coupled nuclease-assisted recycling
25 schemes, which renders a shortened methylene-blue reporter strand that adsorbs on the
26 graphene oxide modified electrode. Adapted with permission from Ref. [68]. (B)
27 Aptasensor using AuNPs to anchor the capture aptamer with two detection modes:
28 “signal-off” voltammetric (upper scheme) and “signal-on” impedimetric measurements
29 (bottom scheme). Adapted with permission from Ref. [76].
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38 Figure 3: Sandwich assays for protein tumor biomarkers using aptamers as a reporting
39 element. (A) Aptasensor for PSA with aptamer for capturing and chronoamperometric
40 detection on disposable electrodes. Reprinted with permission from Ref. [61]. (B)
41 Aptasensor for CA125 using an antibody for capturing and nanoparticles to assist the
42 hybridization chain reaction enabling the amplification of the signal due to the soluble
43 reporter molecule. Adapted with permission from Ref. [72].
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51 Figure 4: Direct aptassays for CTCs detection. (A) “Signal-on” impedimetric cytosensor
52 with subsequent enzyme-based cell recovery. Adapted with permission from Ref. [98].
53 (B) “Signal-off” cytoassays based on a nanochannel-ion channel hybrid. Adapted with
54 permission from Ref. [107].
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Figure 5: Sandwich aptaassays for CTCs detection. (A) Cytosensor based on a DNA walking strategy using AuNPs. Reprinted with permission from Ref. [106]. (B) Dual cytoassay based on nanostructures for capture and signal amplification. Reprinted with permission from Ref. [108]. (C) Voltammetric cytosensor based on nanoprobe carrying HRP-mimicking DNAzymes. Adapted with permission from Ref. [102]. (D) Voltammetric cytosensor based on CuO nanoparticles serving as nanoenzymes. Reprinted with permission from Ref. [101].

Figure 6: Aptasensor designs to detect exosomes. (A) Direct and “signal-off” aptasensor where the aptamer is immobilized through a DNA-nanotetrahedron. Reprinted with permission from Ref. [131]. (B) Displacement design: the recognition of exosomes by the aptamer displaces the hybridized reporter probe, with the subsequent decrease in the signal of the label, methylene blue (MB). Reprinted with permission from Ref. [125]. (C) Immobilized aptamers directly entrap exosomes, which are linked to a reporter probe by a click chemistry process. This probe is amplified in a hybridization chain reaction (HCR) using two biotin-hairpins (biotin-H1 and biotin-H2). The subsequent binding of streptavidin-HRP reports the recognition, after the addition of the substrate of peroxidase, in a “signal-on” response. Reprinted with permission from Ref. [121].

Figure 7: Signal generation and amplifications strategies in sandwich aptasensors to detect exosomes. (A) Dual sensor based on the electrooxidation of metal nanoparticles linked to the reporter aptamers. Reprinted with permission from Ref. [127]. (B) The aptamer is linked with a primer for RCA. After the RCA elongation, a G-quadruplex is formed, which in the presence of hemin is responsible for the voltammetric signal. Reprinted with permission from reference [128]. (C) The interaction between exosomes and the capture receptor (the binding protein Tim 4), immobilized onto magnetic beads, takes place in a microchamber of a microfluidic chip. The beads are magnetically driven to an ITO electrode, where the aptamer elongated with a DNAzyme sequence binds, generating the electrochemical signal in the presence of hemin. Reprinted with permission from reference [124].

1
2 REFERENCES
3

4 [1] S. Gilgunn, P.J. Conroy, R. Saldova, P.M. Rudd, R.J. O'Kennedy, Aberrant PSA
5 glycosylation--a sweet predictor of prostate cancer, *Nat Rev Urol*, 10 (2013) 99-107.
6

7
8
9 [2] C. Tuerk, L. Gold, Systematic evolution of ligands by exponential enrichment -
10 RNA ligands to bacteriophage-T4 DNA-polymerase, *Science*, 249 (1990) 505-510.
11

12
13 [3] A.D. Ellington, J.W. Szostak, Invitro selection of RNA molecules that bind specific
14 ligands, *Nature*, 346 (1990) 818-822.
15

16
17
18 [4] H. Kaur, J.G. Bruno, A. Kumar, T.K. Sharma, Aptamers in the therapeutics and
19 diagnostics pipelines, *Theranostics*, 8 (2018) 4016-4032.
20

21
22
23 [5] D. Xiang, *et al.*, Superior performance of aptamer in tumor penetration over
24 antibody: Implication of aptamer-based theranostics in solid tumors, *Theranostics*, 5
25 (2015) 1083-1097.
26

27
28
29 [6] E.V. Suprun, Protein post-translational modifications – A challenge for
30 bioelectrochemistry, *TRAC Trends Anal Chem*, 116 (2019) 44-60.
31

32
33
34 [7] A.A. Sina, *et al.*, Epigenetically reprogrammed methylation landscape drives the
35 DNA self-assembly and serves as a universal cancer biomarker, *Nat Commun*, 9 (2018)
36 4915.
37

38
39
40 [8] R. Aebersold, *et al.*, How many human proteoforms are there?, *Nat Chem Biol*, 14
41 (2018) 206-214.
42

43
44
45 [9] M. Baker, Reproducibility crisis: Blame it on the antibodies, *Nature*, 521 (2015)
46 274-276.
47

48
49
50 [10] R. O'Kennedy, S. Fitzgerald, C. Murphy, Don't blame it all on antibodies – The
51 need for exhaustive characterisation, appropriate handling, and addressing the issues
52 that affect specificity, *TRAC Trends Anal Chem*, 89 (2017) 53-59.
53

54
55
56 [11] M. Uhlen, *et al.*, A proposal for validation of antibodies, *Nat Methods*, 13 (2016)
57 823-827.
58
59
60
61
62
63
64
65

1 [12] <https://proteomics.cancer.gov/antibody-portal>, December 26, 2019.

2 [13] T.K. Sharma, J.G. Bruno, A. Dhiman, ABCs of DNA aptamer and related assay
3 development, *Biotechnol Adv*, 35 (2017) 275-301.

4 [14] T. Wang, C. Chen, L.M. Larcher, R.A. Barrero, R.N. Veedu, Three decades of
5 nucleic acid aptamer technologies: Lessons learned, progress and opportunities on
6 aptamer development, *Biotechnol Adv*, 37 (2019) 28-50.

7 [15] C. Zong, J. Liu, The arsenic-binding aptamer cannot bind arsenic: Critical
8 evaluation of aptamer selection and binding, *Anal Chem*, 91 (2019) 10887-10893.

9 [16] I. Alvarez-Martos, E.E. Ferapontova, A DNA sequence obtained by replacement of
10 the dopamine RNA aptamer bases is not an aptamer, *Biochem Biophys Res Commun*,
11 489 (2017) 381-385.

12 [17] R. Lorenzo-Gómez, N. Fernández-Alonso, R. Miranda-Castro, N. de-los-Santos-
13 Álvarez, M.J. Lobo-Castañón, Unravelling the lipocalin 2 interaction with aptamers:
14 May rolling circle amplification improve their functional affinity?, *Talanta*, 197 (2019)
15 406-412.

16 [18] K. Ikebukuro, C. Kiyohara, K. Sode, Electrochemical detection of protein using a
17 double aptamer sandwich, *Anal Lett*, 37 (2004) 2901-2909.

18 [19] H. Sun, W. Tan, Y. Zu, Aptamers: versatile molecular recognition probes for
19 cancer detection, *Analyst*, 141 (2016) 403-415.

20 [20] H. Safarpour, S. Dehghani, R. Nosrati, N. Zebardast, M. Alibolandi, A.
21 Mokhtarzadeh, M. Ramezani, Optical and electrochemical-based nano-aptasensing
22 approaches for the detection of circulating tumor cells (CTCs), *Biosens Bioelectron*,
23 148 (2020) 111833.

24 [21] W. Zhou, P.-J. Jimmy Huang, J. Ding, J. Liu, Aptamer-based biosensors for
25 biomedical diagnostics, *Analyst*, 139 (2014) 2627-2640.

26 [22] M. Jarczewska, Ł. Górski, E. Malinowska, Electrochemical aptamer-based
27 biosensors as potential tools for clinical diagnostics, *Anal Methods*, 8 (2016) 3861-
28 3877.

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63
64
65
- [23] M. Hasanzadeh, N. Shadjou, M. de la Guardia, Aptamer-based assay of biomolecules: Recent advances in electro-analytical approach, *TRAC Trends Anal Chem*, 89 (2017) 119-132.
- [24] D. Sadighbayan, K. Sadighbayan, M.R. Tohid-kia, A.Y. Khosroushahi, M. Hasanzadeh, Development of electrochemical biosensors for tumor marker determination towards cancer diagnosis: Recent progress, *TRAC Trends Anal Chem*, 118 (2019) 73-88.
- [25] S.N. Topkaya, M. Azimzadeh, M. Ozsoz, Electrochemical biosensors for cancer biomarkers detection: Recent advances and challenges, *Electroanalysis*, 28 (2016) 1402-1419.
- [26] S. Dehghani, *et al.*, Aptamer-based biosensors and nanosensors for the detection of vascular endothelial growth factor (VEGF): A review, *Biosens Bioelectron*, 110 (2018) 23-37.
- [27] M. Yousefi, *et al.*, Aptasensors as a new sensing technology developed for the detection of MUC1 mucin: A review, *Biosens Bioelectron*, 130 (2019) 1-19.
- [28] P. Jolly, N. Formisano, P. Estrela, DNA aptamer-based detection of prostate cancer, *Chemical Papers*, 69 (2015) 77-89.
- [29] D. Sun, J. Lu, L. Zhang, Z. Chen, Aptamer-based electrochemical cytosensors for tumor cell detection in cancer diagnosis: A review, *Anal Chim Acta*, 1082 (2019) 1-17.
- [30] R. Eivazzadeh-Keihan, P. Pashazadeh-Panahi, B. Baradaran, A. Maleki, M. Hejazi, A. Mokhtarzadeh, M. de la Guardia, Recent advances on nanomaterial based electrochemical and optical aptasensors for detection of cancer biomarkers, *TRAC Trends Anal Chem*, 100 (2018) 103-115.
- [31] Y. Yang, X. Yang, Y. Yang, Q. Yuan, Aptamer-functionalized carbon nanomaterials electrochemical sensors for detecting cancer relevant biomolecules, *Carbon*, 129 (2018) 380-395.

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64
65
- [32] E.M. Hassan, M.C. DeRosa, Recent advances in cancer early detection and diagnosis: Role of nucleic acid based aptasensors, *TRAC Trends Anal Chem*, 124 (2020) 115806.
- [33] R. Miranda-Castro, I. Palchetti, N. de-los-Santos-Álvarez, The Translational Potential of Electrochemical DNA-Based Liquid Biopsy, *Frontiers in Chemistry*, 8 (2020).
- [34] E.J. Cho, J.W. Lee, A.D. Ellington, Applications of aptamers as sensors, *Annu Rev Anal Chem*, 2 (2009) 241-264.
- [35] Z. Tang, *et al.*, Selection of aptamers for molecular recognition and characterization of cancer cells, *Anal Chem*, 79 (2007) 4900-4907.
- [36] S. Goto, K. Tsukakoshi, K. Ikebukuro, Development of aptamers against unpurified proteins, *Biotechnol Bioeng*, 114 (2017) 2706-2716.
- [37] Y. Zheng, *et al.*, DNA aptamers from whole-serum SELEX as new diagnostic agents against gastric cancer, *RSC Advances*, 9 (2019) 950-957.
- [38] K. Li, L. Qi, L. Gao, M. Shi, J. Li, Z. Liu, L. Zhao, Selection and preliminary application of a single stranded DNA aptamer targeting colorectal cancer serum, *RSC Advances*, 9 (2019) 38867-38876.
- [39] T. Wang, W.-X. Chen, G.-F. Xu, B. Zhang, S.-Q. Wei, J. Hu, K.-H. Zhang, A universal strategy designed for selecting bench-to-bedside aptamers to serum and validated in hepatocellular carcinoma diagnosis, *Microchem J*, 150 (2019) 104152.
- [40] J.G. Bruno, Effects of various additives on cancer biomarker aptamer-magnetic pull-down in human serum, *J Bionanosci*, 11 (2017) 45-51.
- [41] G.S. Zamay, *et al.*, Aptamers selected to postoperative lung adenocarcinoma detect circulating tumor cells in human blood, *Mol Ther*, 23 (2015) 1486-1496.
- [42] G.S. Zamay, *et al.*, Electrochemical aptasensor for lung cancer-related protein detection in crude blood plasma samples, *Sci Rep*, 6 (2016) 34350.

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60
61
62
63
64
65
- [43] S. Li, *et al.*, Identification of an aptamer targeting hnRNP A1 by tissue slide-based SELEX, *J Pathol*, 218 (2009) 327-336.
- [44] H. Wang, *et al.*, Morph-X-Select: Morphology-based tissue aptamer selection for ovarian cancer biomarker discovery, *BioTechniques*, 61 (2016) 249-259.
- [45] W.-T. Liu, W.-B. Lee, Y.-C. Tsai, Y.-J. Chuang, K.-F. Hsu, G.-B. Lee, An automated microfluidic system for selection of aptamer probes against ovarian cancer tissues, *Biomicrofluidics*, 13 (2019) 014114.
- [46] W. Zhong, *et al.*, Identification and application of an aptamer targeting papillary thyroid carcinoma using tissue-SELEX, *Anal Chem*, 91 (2019) 8289-8297.
- [47] J. Mi, Y. Liu, Z.N. Rabbani, Z. Yang, J.H. Urban, B.A. Sullenger, B.M. Clary, In vivo selection of tumor-targeting RNA motifs, *Nat Chem Biol*, 6 (2010) 22-24.
- [48] J. Mi, *et al.*, In vivo selection against human colorectal cancer xenografts identifies an aptamer that targets RNA helicase protein DHX9, *Mol Ther Nucleic Acids*, 5 (2016) e315.
- [49] H. Wang, Y. Zhang, H. Yang, M. Qin, X. Ding, R. Liu, Y. Jiang, In vivo SELEX of an inhibitory NSCLC-specific RNA aptamer from PEGylated RNA library, *Mol Ther Nucleic Acids*, 10 (2018) 187-198.
- [50] L. Civit, *et al.*, Targeting hormone refractory prostate cancer by in vivo selected DNA libraries in an orthotopic xenograft mouse model, *Sci Rep*, 9 (2019) 4976.
- [51] L. Chen, *et al.*, In vivo SELEX of bone targeting aptamer in prostate cancer bone metastasis model, *Int J Nanomedicine*, 14 (2018) 149-159.
- [52] J.-J. Zhang, J.-T. Cao, G.-F. Shi, K.-J. Huang, Y.-M. Liu, S.-W. Ren, A luminol electrochemiluminescence aptasensor based on glucose oxidase modified gold nanoparticles for measurement of platelet-derived growth factor BB, *Talanta*, 132 (2015) 65-71.
- [53] X. Liu, H.-L. Shuai, K.-J. Huang, A label-free electrochemical aptasensor based on leaf-like vanadium disulfide-Au nanoparticles for the sensitive and selective detection of platelet-derived growth factor BB, *Anal Methods*, 7 (2015) 8277-8284.

1 [54] Z. Hao, Y. Pan, W. Shao, Q. Lin, X. Zhao, Graphene-based fully integrated
2 portable nanosensing system for on-line detection of cytokine biomarkers in saliva,
3 Biosens Bioelectron, 134 (2019) 16-23.
4
5

6 [55] M. Heiat, M. Negahdary, Sensitive diagnosis of alpha-fetoprotein by a label free
7 nanoaptasensor designed by modified Au electrode with spindle-shaped gold
8 nanostructure, Microchem J, 148 (2019) 456-466.
9
10

11 [56] M. Cui, Y. Wang, M. Jiao, S. Jayachandran, Y. Wu, X. Fan, X. Luo, Mixed self-
12 assembled aptamer and newly designed zwitterionic peptide as antifouling biosensing
13 interface for electrochemical detection of alpha-fetoprotein, ACS Sens, 2 (2017) 490-
14 494.
15
16

17 [57] A. Rahi, N. Sattarahmady, H. Heli, Label-free electrochemical aptasensing of the
18 human prostate-specific antigen using gold nanospears, Talanta, 156-157 (2016) 218-
19 224.
20
21

22 [58] E. Heydari-Bafrooei, N.S. Shamszadeh, Electrochemical bioassay development for
23 ultrasensitive aptasensing of prostate specific antigen, Biosens Bioelectron, 91 (2017)
24 284-292.
25
26

27 [59] B. Wei, K. Mao, N. Liu, M. Zhang, Z. Yang, Graphene nanocomposites modified
28 electrochemical aptamer sensor for rapid and highly sensitive detection of prostate
29 specific antigen, Biosens Bioelectron, 121 (2018) 41-46.
30
31

32 [60] N. Sattarahmady, A. Rahi, H. Heli, A signal-on built in-marker electrochemical
33 aptasensor for human prostate-specific antigen based on a hairbrush-like gold
34 nanostructure, Sci Rep, 7 (2017) 11238.
35
36

37 [61] A. Díaz-Fernández, R. Miranda-Castro, N. de-los-Santos-Álvarez, E.F. Rodríguez,
38 M.J. Lobo-Castañón, Focusing aptamer selection on the glycan structure of prostate-
39 specific antigen: Toward more specific detection of prostate cancer, Biosens
40 Bioelectron, 128 (2019) 83-90.
41
42

43 [62] C. Ma, H. Liu, L. Zhang, H. Li, M. Yan, X. Song, J. Yu, Multiplexed aptasensor
44 for simultaneous detection of carcinoembryonic antigen and mucin-1 based on metal ion
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 electrochemical labels and $\text{Ru}(\text{NH}_3)_6^{3+}$ electronic wires, *Biosens Bioelectron*, 99 (2018)
2 8-13.
3

4 [63] G. Paniagua, *et al.*, Amperometric aptasensor for carcinoembryonic antigen based
5 on the use of bifunctionalized Janus nanoparticles as biorecognition-signaling element,
6 *Anal Chim Acta*, 1061 (2019) 84-91.
7
8
9

10 [64] W. Wen, *et al.*, Increased electrocatalyzed performance through hairpin
11 oligonucleotide aptamer-functionalized gold nanorods labels and graphene-streptavidin
12 nanomatrix: Highly selective and sensitive electrochemical biosensor of
13 carcinoembryonic antigen, *Biosens Bioelectron*, 83 (2016) 142-148.
14
15
16
17
18

19 [65] J.-J. Yang, J.-T. Cao, Y.-L. Wang, H. Wang, Y.-M. Liu, S.-H. Ma, Sandwich-like
20 electrochemiluminescence aptasensor based on dual quenching effect from hemin-
21 graphene nanosheet and enzymatic biocatalytic precipitation for sensitive detection of
22 carcinoembryonic antigen, *J Electroanal Chem*, 787 (2017) 88-94.
23
24
25
26
27

28 [66] Z. Liu, S. Lei, L. Zou, G. Li, L. Xu, B. Ye, A label-free and double recognition-
29 amplification novel strategy for sensitive and accurate carcinoembryonic antigen assay,
30 *Biosens Bioelectron*, 131 (2019) 113-118.
31
32
33

34 [67] Y. Wang, *et al.*, Label-free microfluidic paper-based electrochemical aptasensor for
35 ultrasensitive and simultaneous multiplexed detection of cancer biomarkers, *Biosens*
36 *Bioelectron*, 136 (2019) 84-90.
37
38
39
40

41 [68] L. Ge, W. Wang, X. Sun, T. Hou, F. Li, Affinity-mediated homogeneous
42 electrochemical aptasensor on a graphene platform for ultrasensitive biomolecule
43 detection via exonuclease-assisted target-analog recycling amplification, *Anal Chem*, 88
44 (2016) 2212-2219.
45
46
47
48

49 [69] W. Jiang, L. Liu, L. Zhang, Q. Guo, Y. Cui, M. Yang, Sensitive immunosensing of
50 the carcinoembryonic antigen utilizing aptamer-based in-situ formation of a redox-
51 active heteropolyacid and rolling circle amplification, *Microchim Acta*, 184 (2017)
52 4757-4763.
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[70] K. Malecka, D. Pankratov, E.E. Ferapontova, Femtomolar electroanalysis of a breast cancer biomarker HER-2/neu protein in human serum by the cellulase-linked sandwich assay on magnetic beads, *Anal Chim Acta*, 1077 (2019) 140-149.

[71] C. Shen, K. Zeng, J. Luo, X. Li, M. Yang, A. Rasooly, Self-assembled DNA generated electric current biosensor for HER2 analysis, *Anal Chem*, 89 (2017) 10264-10269.

[72] Y. Nie, M. Yang, Y. Ding, Gold nanoparticle enhanced hybridization chain reaction as a method for signal amplification. Application to electrochemical immunodetection of the ovarian cancer biomarker carbohydrate antigen 125, *Microchim Acta*, 185 (2018) 331.

[73] L. Farzin, S. Sadjadi, M. Shamsipur, S. Sheibani, M.h. Mousazadeh, Employing AgNPs doped amidoxime-modified polyacrylonitrile (PAN-oxime) nanofibers for target induced strand displacement-based electrochemical aptasensing of CA125 in ovarian cancer patients, *Mater Sci Eng C*, 97 (2019) 679-687.

[74] S. Mansouri Majd, A. Salimi, Ultrasensitive flexible FET-type aptasensor for CA 125 cancer marker detection based on carboxylated multiwalled carbon nanotubes immobilized onto reduced graphene oxide film, *Anal Chim Acta*, 1000 (2018) 273-282.

[75] S.G. Meirinho, L.G. Dias, A.M. Peres, L.R. Rodrigues, Electrochemical aptasensor for human osteopontin detection using a DNA aptamer selected by SELEX, *Anal Chim Acta*, 987 (2017) 25-37.

[76] M. Shamsipur, L. Farzin, M. Amouzadeh Tabrizi, F. Molaabasi, Highly sensitive label free electrochemical detection of VEGF165 tumor marker based on “signal off” and “signal on” strategies using an anti-VEGF165 aptamer immobilized BSA-gold nanoclusters/ionic liquid/glassy carbon electrode, *Biosens Bioelectron*, 74 (2015) 369-375.

[77] M. Amouzadeh Tabrizi, M. Shamsipur, L. Farzin, A high sensitive electrochemical aptasensor for the determination of VEGF165 in serum of lung cancer patient, *Biosens Bioelectron*, 74 (2015) 764-769.

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47
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58
59
60
61
62
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65
- [78] H. Ilkhani, M. Sarparast, A. Noori, S. Zahra Bathaie, M.F. Mousavi, Electrochemical aptamer/antibody based sandwich immunosensor for the detection of EGFR, a cancer biomarker, using gold nanoparticles as a signaling probe, *Biosens Bioelectron*, 74 (2015) 491-497.
- [79] C.S. Ferreira, C.S. Matthews, S. Missailidis, DNA aptamers that bind to MUC1 tumour marker: design and characterization of MUC1-binding single-stranded DNA aptamers, *Tumour Biol*, 27 (2006) 289-301.
- [80] C.S.M. Ferreira, K. Papamichael, G. Guilbault, T. Schwarzacher, J. Gariépy, S. Missailidis, DNA aptamers against the MUC1 tumour marker: design of aptamer–antibody sandwich ELISA for the early diagnosis of epithelial tumours, *Anal Bioanal Chem*, 390 (2008) 1039-1050.
- [81] Z. Mi, H. Guo, P.C. Kuo, Identification of osteopontin-dependent signaling pathways in a mouse model of human breast cancer, *BMC Research Notes*, 2 (2009) 119.
- [82] S.G. Meirinho, L.G. Dias, A.M. Peres, L.R. Rodrigues, Development of an electrochemical RNA-aptasensor to detect human osteopontin, *Biosens Bioelectron*, 71 (2015) 332-341.
- [83] F. Kuralay, S. Campuzano, J. Wang, Greatly extended storage stability of electrochemical DNA biosensors using ternary thiolated self-assembled monolayers, *Talanta*, 99 (2012) 155-160.
- [84] S. Campuzano, *et al.*, Ternary monolayers as DNA recognition interfaces for direct and sensitive electrochemical detection in untreated clinical samples, *Biosens Bioelectron*, 26 (2011) 3577-3583.
- [85] R.Y. Lai, K.W. Plaxco, A.J. Heeger, Aptamer-based electrochemical detection of picomolar platelet-derived growth factor directly in blood serum, *Anal Chem*, 79 (2007) 229-233.
- [86] L.R. Schoukroun-Barnes, F.C. Macazo, B. Gutierrez, J. Lottermoser, J. Liu, R.J. White, Reagentless, structure-switching, electrochemical aptamer-based sensors, *Annu Rev Anal Chem*, 9 (2016) 163-181.

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65
- [87] M.A. Pellitero, A. Shaver, N. Arroyo-Currás, Critical Review—Approaches for the Electrochemical Interrogation of DNA-Based Sensors: A Critical Review, *J Electrochem Soc*, 167 (2020) 037529.
- [88] A. Díaz-Fernández, R. Miranda-Castro, N. Díaz, D. Suárez, N. de-los-Santos-Alvarez, M.J. Lobo-Castañón, Aptamers targeting protein-specific glycosylation in tumor biomarkers: general selection, characterization and structural modeling, (2020 submitted).
- [89] C.Q. Vu, P. Rotkrua, Y. Tantirungrotechai, B. Soontornworajit, Oligonucleotide hybridization combined with competitive antibody binding for the truncation of a high-affinity aptamer, *ACS Combi Sci*, 19 (2017) 609-617.
- [90] L.S. Green, Inhibitory DNA ligands to platelet-derived growth factor B-chain, *Biochemistry-U.S.*, 35 (1996) 14413-14424.
- [91] G. Mahlknecht, R. Maron, M. Mancini, B. Schechter, M. Sela, Y. Yarden, Aptamer to ErbB-2/HER2 enhances degradation of the target and inhibits tumorigenic growth, *Proc Natl Acad Sci*, 110 (2013) 8170-8175.
- [92] J.D. Cohen, *et al.*, Detection and localization of surgically resectable cancers with a multi-analyte blood test, *Science*, 359 (2018) 926-930.
- [93] N. Savory, K. Abe, K. Sode, K. Ikebukuro, Selection of DNA aptamer against prostate specific antigen using a genetic algorithm and application to sensing, *Biosens Bioelectron*, 26 (2010) 1386-1391.
- [94] C.L. Chaffer, R.A. Weinberg, A perspective on cancer cell metastasis, *Science*, 331 (2011) 1559-1564.
- [95] Z. Shen, A. Wu, X. Chen, Current detection technologies for circulating tumor cells, *Chem Soc Rev*, 46 (2017) 2038-2056.
- [96] Y. Zhao, D. Xu, W. Tan, Aptamer-functionalized nano/micro-materials for clinical diagnosis: isolation, release and bioanalysis of circulating tumor cells, *Integr Biol*, 9 (2017) 188-205.

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65
- [97] Y. Song, *et al.*, Selection of DNA aptamers against epithelial cell adhesion molecule for cancer cell imaging and circulating tumor cell capture, *Anal Chem*, 85 (2013) 4141-4149.
- [98] H. Shen, *et al.*, A novel label-free and reusable electrochemical cytosensor for highly sensitive detection and specific collection of CTCs, *Biosens Bioelectron*, 81 (2016) 495-502.
- [99] C. Shen, S. Liu, X. Li, M. Yang, Electrochemical detection of circulating tumor cells based on DNA generated electrochemical current and Rolling Circle Amplification, *Anal Chem*, 91 (2019) 11614-11619.
- [100] J.-X. Liu, N. Bao, X. Luo, S.-N. Ding, Nonenzymatic amperometric aptamer cytosensor for ultrasensitive detection of circulating tumor cells and dynamic evaluation of cell surface N-glycan expression, *ACS Omega*, 3 (2018) 8595-8604.
- [101] L. Tian, *et al.*, Copper (II) oxide nanozyme based electrochemical cytosensor for high sensitive detection of circulating tumor cells in breast cancer, *J Electroanal Chem*, 812 (2018) 1-9.
- [102] D. Ou, D. Sun, Z. Liang, B. Chen, X. Lin, Z. Chen, A novel cytosensor for capture, detection and release of breast cancer cells based on metal organic framework PCN-224 and DNA tetrahedron linked dual-aptamer, *Sens Actuat B-Chem*, 285 (2019) 398-404.
- [103] D. Shangguan, *et al.*, Aptamers evolved from live cells as effective molecular probes for cancer study, *Proc Natl Acad Sci*, 103 (2006) 11838-11843.
- [104] D. Shangguan, Z. Tang, P. Mallikaratchy, Z. Xiao, W. Tan, Optimization and modifications of aptamers selected from live cancer cell lines, *Chembiochem*, 8 (2007) 603-606.
- [105] S.-S. Wang, X.-P. Zhao, F.-F. Liu, M.R. Younis, X.-H. Xia, C. Wang, Direct plasmon-enhanced electrochemistry for enabling ultrasensitive and label-free detection of circulating tumor cells in blood, *Anal Chem*, 91 (2019) 4413-4420.

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- [106] P. Miao, Y. Tang, Gold nanoparticles-based multipedal DNA walker for ratiometric detection of circulating tumor cell, *Anal Chem*, 91 (2019) 15187-15192.
- [107] J. Cao, X.-P. Zhao, M.R. Younis, Z.-Q. Li, X.-H. Xia, C. Wang, Ultrasensitive capture, detection, and release of circulating tumor cells using a nanochannel–ion channel hybrid coupled with electrochemical detection technique, *Anal Chem*, 89 (2017) 10957-10964.
- [108] B. Dou, L. Xu, B. Jiang, R. Yuan, Y. Xiang, Aptamer-functionalized and gold nanoparticle array-decorated magnetic graphene nanosheets enable multiplexed and sensitive electrochemical detection of rare circulating tumor cells in whole blood, *Anal Chem*, 91 (2019) 10792-10799.
- [109] P. Mallikaratchy, Z. Tang, S. Kwame, L. Meng, D. Shangguan, W. Tan, Aptamer directly evolved from live cells recognizes membrane bound immunoglobulin heavy mu chain in Burkitt's lymphoma cells, *Mol Cell Proteomics*, 6 (2007) 2230-2238.
- [110] D. Sun, J. Lu, Z. Luo, L. Zhang, P. Liu, Z. Chen, Competitive electrochemical platform for ultrasensitive cytosensing of liver cancer cells by using nanotetrahedra structure with rolling circle amplification, *Biosens Bioelectron*, 120 (2018) 8-14.
- [111] L. Qu, J. Xu, X. Tan, Z. Liu, L. Xu, R. Peng, Dual-aptamer Modification generates a unique interface for highly sensitive and specific electrochemical detection of tumor cells, *ACS Appl Mater Interfaces*, 6 (2014) 7309-7315.
- [112] F. Li, S. Hu, R. Zhang, Y. Gu, Y. Li, Y. Jia, Porous graphene oxide enhanced aptamer specific circulating-tumor-cell sensing interface on light addressable potentiometric sensor: Clinical application and simulation, *ACS Appl Mater Interfaces*, 11 (2019) 8704-8709.
- [113] D.R. Thévenot, K. Toth, R.A. Durst, G.S. Wilson, Electrochemical biosensors: Recommended definitions and classification, *Pure Appl Chem*, 71 (1999) 2333-2348.
- [114] R. Xu, A. Rai, M. Chen, W. Suwakulsiri, D.W. Greening, R.J. Simpson, Extracellular vesicles in cancer — implications for future improvements in cancer care, *Nat Rev Clin Oncol*, 15 (2018) 617-638.

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62
63
64
65
- [115] J.-H. Kim, E. Kim, M.Y. Lee, Exosomes as diagnostic biomarkers in cancer, *Mol Cell Toxicol*, 14 (2018) 113-122.
- [116] M.F. Peterson, N. Otoc, J.K. Sethi, A. Gupta, T.J. Antes, Integrated systems for exosome investigation, *Methods*, 87 (2015) 31-45.
- [117] B.S. Chia, Y.P. Low, Q. Wang, P. Li, Z. Gao, Advances in exosome quantification techniques, *TRAC Trends Anal Chem*, 86 (2017) 93-106.
- [118] H. Zhang, Z. Wang, Q. Zhang, F. Wang, Y. Liu, Ti₃C₂ MXenes nanosheets catalyzed highly efficient electrogenerated chemiluminescence biosensor for the detection of exosomes, *Biosens Bioelectron*, 124-125 (2019) 184-190.
- [119] L. Zhao, R. Sun, P. He, X. Zhang, Ultrasensitive detection of exosomes by target-triggered three-dimensional DNA walking machine and exonuclease III-assisted electrochemical ratiometric biosensing, *Anal Chem*, 91 (2019) 14773-14779.
- [120] X. Yin, T. Hou, B. Huang, L. Yang, F. Li, Aptamer recognition-triggered label-free homogeneous electrochemical strategy for an ultrasensitive cancer-derived exosome assay, *Chem Commun*, 55 (2019) 13705-13708.
- [121] Y. An, T. Jin, Y. Zhu, F. Zhang, P. He, An ultrasensitive electrochemical aptasensor for the determination of tumor exosomes based on click chemistry, *Biosens Bioelectron*, 142 (2019) 111503.
- [122] B. Qiao, *et al.*, An electrochemiluminescent aptasensor for amplified detection of exosomes from breast tumor cells (MCF-7 cells) based on G-quadruplex/hemin DNazymes, *Analyst*, 144 (2019) 3668-3675.
- [123] Y. Cao, L. Li, B. Han, Y. Wang, Y. Dai, J. Zhao, A catalytic molecule machine-driven biosensing method for amplified electrochemical detection of exosomes, *Biosens Bioelectron*, 141 (2019) 111397.
- [124] H. Xu, C. Liao, P. Zuo, Z. Liu, B.-C. Ye, Magnetic-based microfluidic device for on-chip isolation and detection of tumor-derived exosomes, *Anal Chem*, 90 (2018) 13451-13458.

1 [125] Q. Zhou, A. Rahimian, K. Son, D.-S. Shin, T. Patel, A. Revzin, Development of
2 an aptasensor for electrochemical detection of exosomes, *Methods*, 97 (2016) 88-93.
3

4 [126] <https://www.basepairbio.com/>, December 23, 2019.
5
6

7 [127] Y.-G. Zhou, *et al.*, Interrogating circulating microsomes and exosomes using
8 metal nanoparticles, *Small*, 12 (2016) 727-732.
9

10 [128] R. Huang, *et al.*, A sensitive aptasensor based on a hemin/G-quadruplex-assisted
11 signal amplification strategy for electrochemical detection of gastric cancer exosomes,
12 *Small*, 15 (2019) e1900735.
13
14
15
16

17 [129] H. Dong, H. Chen, J. Jiang, H. Zhang, C. Cai, Q. Shen, Highly sensitive
18 electrochemical detection of tumor exosomes based on aptamer recognition-induced
19 multi-DNA release and cyclic enzymatic amplification, *Anal Chem*, 90 (2018) 4507-
20 4513.
21
22
23
24
25
26

27 [130] L. Zhang, *et al.*, Evolution of functional six-nucleotide DNA, *J Am Chem Soc*,
28 137 (2015) 6734-6737.
29
30

31 [131] S. Wang, *et al.*, Aptasensor with expanded nucleotide using DNA nanotetrahedra
32 for electrochemical detection of cancerous exosomes, *ACS Nano*, 11 (2017) 3943-3949.
33
34
35

36 [132] F. Wei, J. Yang, D.T. Wong, Detection of exosomal biomarker by electric field-
37 induced release and measurement (EFIRM), *Biosens Bioelectron*, 44 (2013) 115-121.
38
39
40

41 [133] H. Katoh, K. Nakamura, T. Tanaka, S. Satomura, S. Matsuura, Automatic and
42 simultaneous analysis of *Lens culinaris* agglutinin-reactive α -fetoprotein ratio and total
43 α -fetoprotein concentration, *Anal Chem*, 70 (1998) 2110-2114.
44
45
46

47 [134] A.R. Horvath, *et al.*, From biomarkers to medical tests: The changing landscape
48 of test evaluation, *Clin Chim Acta*, 427 (2014) 49-57.
49
50
51

52 [135] M. Duan, *et al.*, Selection and characterization of DNA aptamer for metastatic
53 prostate cancer recognition and tissue imaging, *Oncotarget*, 7 (2016).
54
55
56
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57
58
59
60
61
62
63
64
65
- [136] W. Sheng, T. Chen, W. Tan, Z.H. Fan, Multivalent DNA nanospheres for enhanced capture of cancer cells in microfluidic devices, *ACS Nano*, 7 (2013) 7067-7076.
- [137] W. Zhao, *et al.*, Bioinspired multivalent DNA network for capture and release of cells, *Proc Natl Acad Sci*, (2012) 201211234.
- [138] <http://apollox.com/investors/>, December 28, 2019.
- [139] J.-F. Chen, *et al.*, Clinical applications of NanoVelcro rare-cell assays for detection and characterization of circulating tumor cells, *Theranostics*, 6 (2016) 1425-1439.
- [140] <https://patentscope.wipo.int/search/en/search.jsf>, April 7, 2020.
- [141] S. Angione, M. Cooper, J. Coppeta, T. Mulhern, H. Azizgolshani, Regeneratable biosensor and methods of use thereof, EP3548891, 2019
- [142] W. Fang, L. Wei, Probe immobilization and signal amplification for polymer-based biosensor, US20100330706, 2015
- [143] F. Chunhai, Y. Fan, Z. Xiaolei, H. Qing, Integrated type microfluidic electrochemical biosensor system and method for rapid biochemical analysis, US20150153300, 2015
- [144] M.B. Kjelstrup, C. Hejesen, K.V. Gothelf, Z. Zhang, Detection of non-nucleic acid analytes using strand displacement exchange reactions, EP2895617, 2015
- [145] M.B. Gu, J.W. Park, T. Rameshwar, Aptamers screening method based on graphene without target immobilization and the aptamers obtained from the method, US20140363808, 2016
- [146] C. Ban, Y.-K. Kim, H. Jo, S. Lee, H.J. Youn, DNA aptamer specifically binding to EN2 (ENGRAILED-2) and use thereof, US20160187342, 2016
- [147] W.R. Pagels, Systems and methods for diagnostic testing, US20150268186, 2015
- [148] S.J. Pearton, T. Lele, Sensors using high electron mobility transistors, US20110074381, 2014

1 [149] J. Lotvall, J. S.C., R. Crescitelli, Tissue-derived extracellular vesicles and their
2 use as diagnostics, GB2560897 2018
3

4 [150] D. Halbert, V. Domenyuk, D. Spetzler, T. Hornung, F. Schafer, N. Xiao,
5 Aptamers and uses thereof, US20190317099, 2019
6
7

8
9 [151] C. Hui, Aptamer for the capture, diagnosis, enumeration, and eradication of
10 circulating tumor cells US20130035630 2013
11
12

13 [152] G.A. Martin, S. Soper, M. Witek, J.J. Yeh, In vitro capture and analysis of
14 circulating tumor cells, EP2919910, 2015
15
16
17
18
19
20
21
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Fig. 2

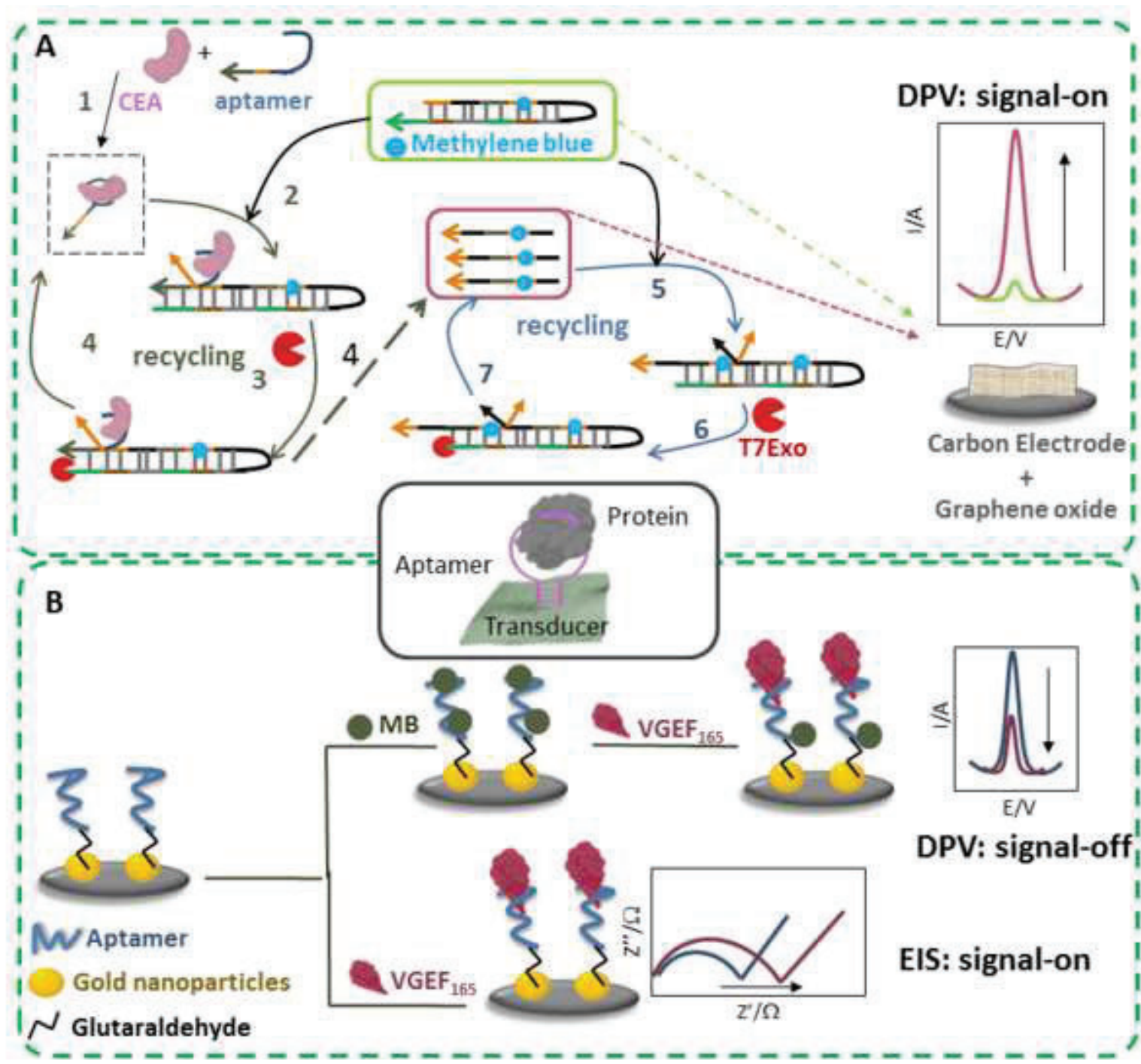


Figure 3

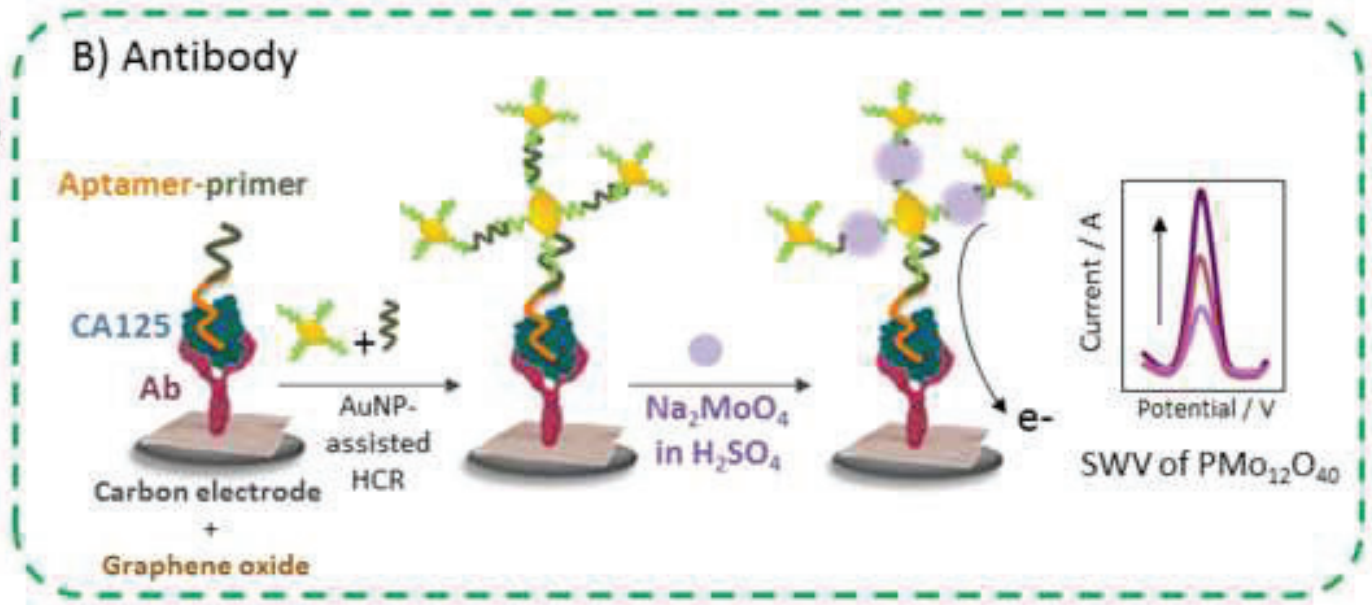
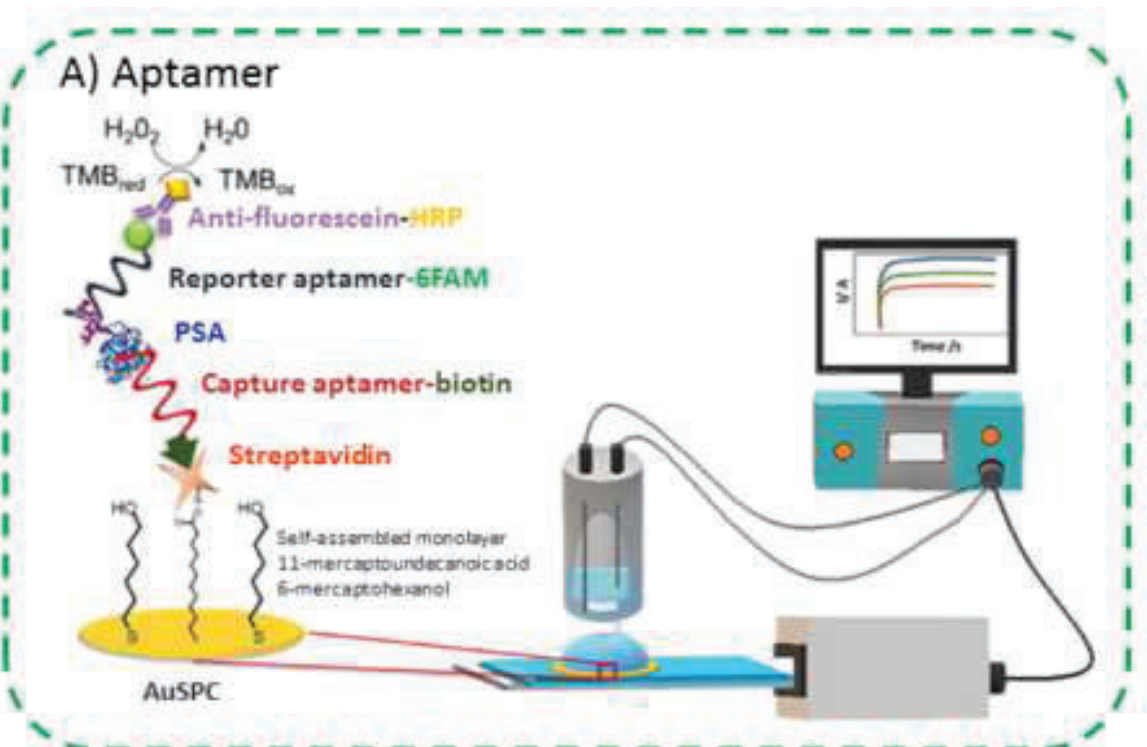
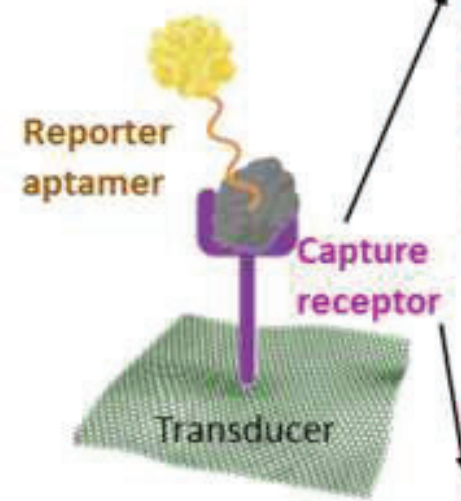
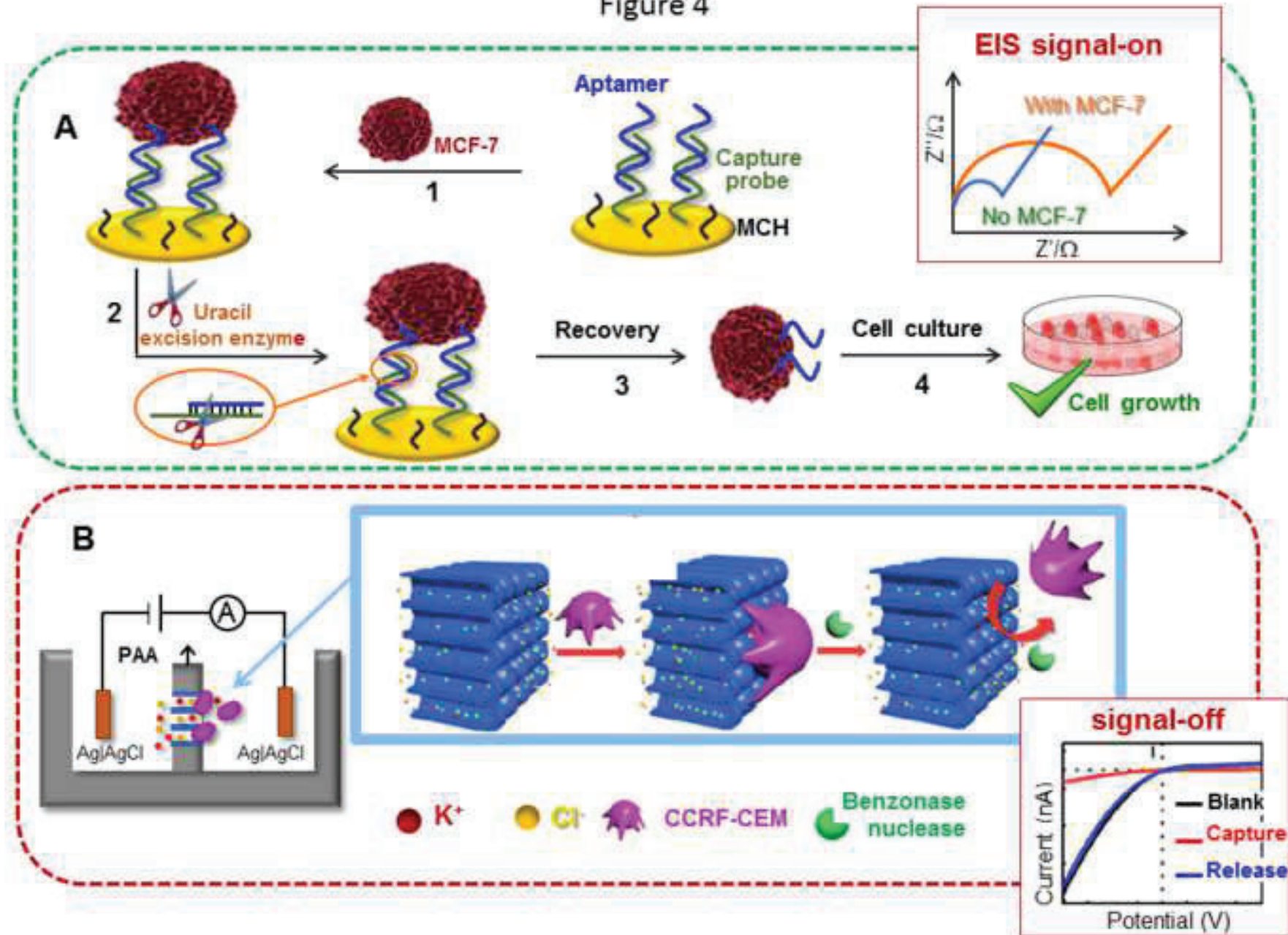
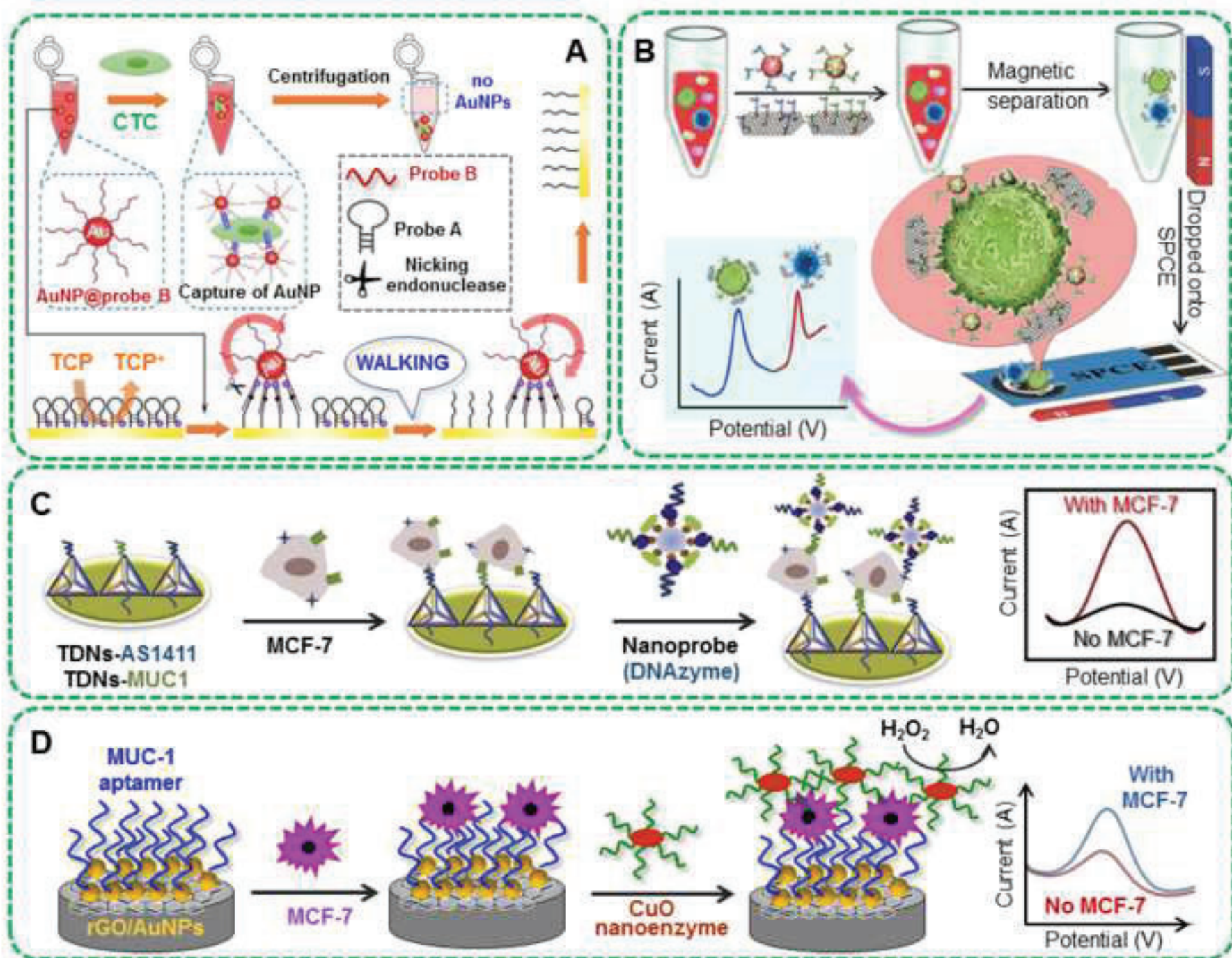


Figure 4



Figure

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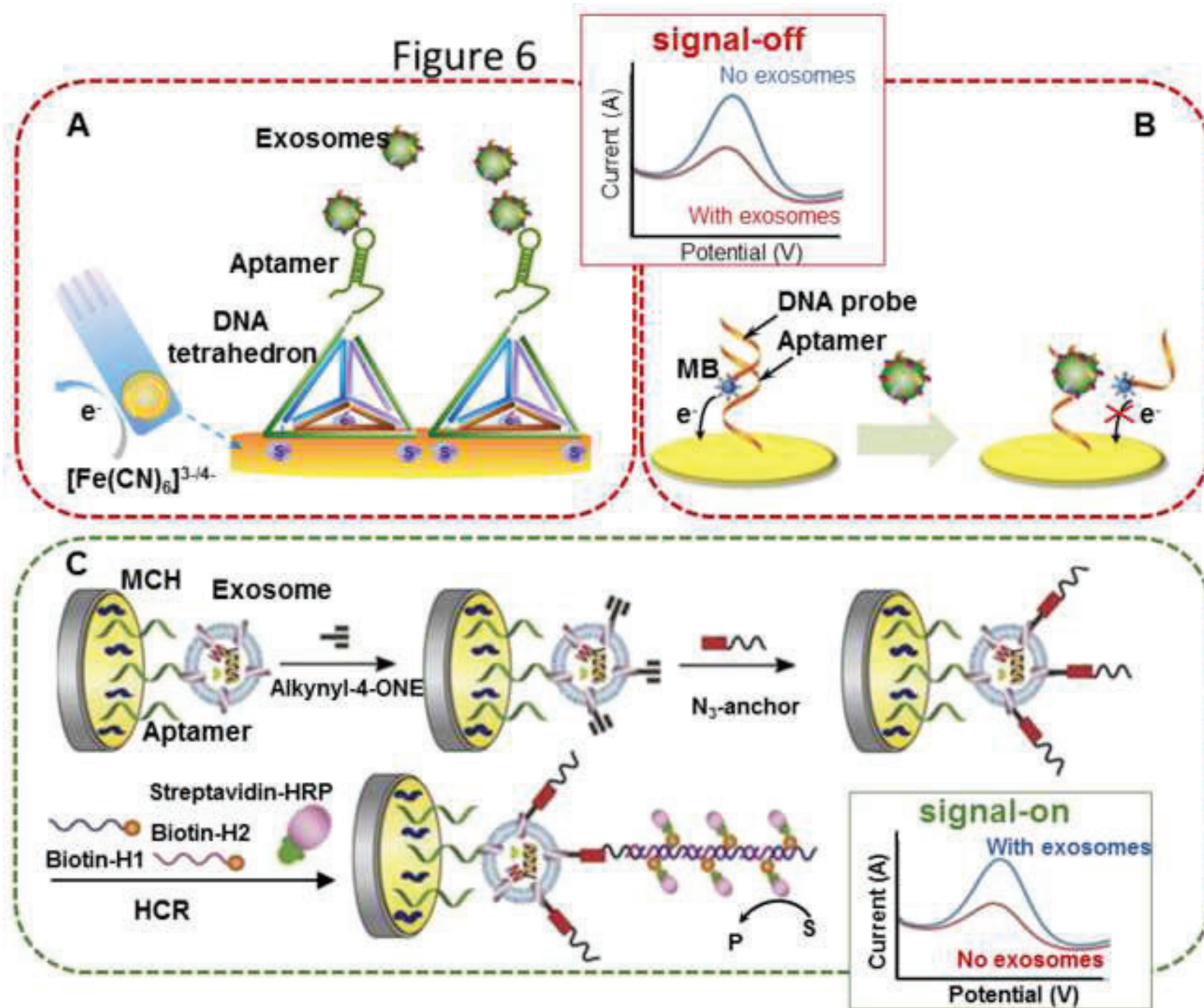


Figure 7

