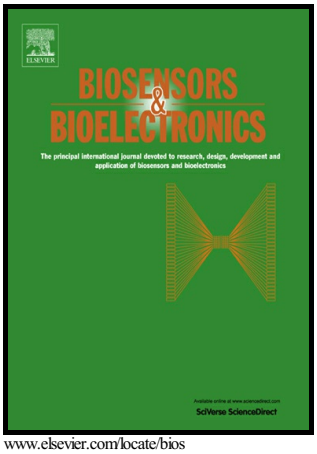

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Nano-biosensing approaches on tuberculosis: defy of aptamers

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Abstract

Tuberculosis is a major global health problem caused by the bacterium *Mycobacterium tuberculosis* (*Mtb*) complex. According to WHO reports, 53 million TB patients died from 2000 to 2016. Therefore, early diagnosis of the disease is of great importance for global health care programs. The restrictions of traditional methods have encouraged the development of innovative methods for rapid, reliable, and cost-effective diagnosis of tuberculosis. In recent years, aptamer-based biosensors or aptasensors have drawn great attention to sensitive and accessible detection of tuberculosis. Aptamers are small short single-stranded molecules of DNA or RNA that fold to a unique form and bind to targets. Once combined with nanomaterials, nano-scale aptasensors provide powerful analytical platforms for diagnosing of tuberculosis. Various groups designed and studied aptamers specific for the whole cells of *M. tuberculosis*, mycobacterial proteins and IFN-γ for early diagnosis of TB. Advantages such as high specificity and strong affinity, potential for binding to a larger variety of targets, increased stability, lower costs of synthesis and storage requirements, and lower probability of contamination make aptasensors pivotal alternatives for future TB diagnostics. In recent years, the concept of SOMAmer has opened new horizons in high precision detection of tuberculosis biomarkers. This review article provides a description of the research progresses of aptamer-based and SOMAmer-based biosensors and nanobiosensors for the detection of tuberculosis.

Keywords: Aptamer, Aptasensor, Biosensor, SOMAmer, SOMAscan, *Mycobacterium tuberculosis*

1. Introduction

Tuberculosis (TB) is one of the most catastrophic diseases that human history ever witnessed. It is a bacterial disease caused by transmission of a member of the *Mycobacterium tuberculosis* complex (MTBC) bacteria (Comas et al. 2013; Faddoul 2015). The latent TB infection (LTBI) is the dormant

form of *Mtb* (viable but functionally inactive) and is considered as a nonclinical TB case. It is estimated that 5 to 10 % of LTBI cases progress toward active TB, a process called "TB reactivation" (Cayabyab et al. 2012). In the 2017 global tuberculosis report, World Health Organization (WHO) estimated that 6.3 million people had developed TB and 53 million of the patients perished due to the disease between the years 2000 to 2016. The report also indicated that about 83 percent of the 5.9 million newly diagnosed and relapsed patients were treated through effective diagnosis and management in 2015 (Organization 2017). Therefore, fast detection and characterization of MTBC is critically important and vital for controlling the TB infections. Moreover, it is crucial to control the spread of the disease and to avoid multidrug-resistant (MDR)-TB (Gopinath et al. 2014).

There are several traditional detection and quantification methods based on the detection of *Mtb* in sputum including culture-colony counting (gold standard), PCR-based, and antigen-antibody interaction methods (Goletti et al. 2016; Lazcka et al. 2007). However, culture-based diagnosis is time-consuming (Goletti et al. 2016) and nucleic acid-based methods (PCR, RT-PCR and real-time PCR) often require sophisticated instrumentations with complicated protocols which make them expensive, labor-intensive, and curb their use in real-time clinical diagnoses (Haas et al. 2016; Nour-Neamatollahi et al. 2018). The reactive tuberculin skin testing (TST) and/or interferon-gamma release assays (IGRAs) methods are used for diagnosis of LTBI (Salgame et al. 2015). The TST and peripheral blood interferon-γ release assays do not distinguish active TB from a cleared or latent infection (Haas et al. 2016; Pai et al. 2014). Although, the Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat (MIRU-VNTR) technique is a more sensitive and fast genotyping method for detection of the tuberculosis mixed infection but this method is very expensive (Tarashi et al. 2017). Recently, Gene-Xpert MTB/RIF has been introduced as a rapid sputum molecular diagnostic test for TB detection. This method works very well, except perhaps in smear-negative TB and pediatric TB cases (Ozkutuk and Surucüoglu 2014; Singh et al. 2016).

The development of a rapid, highly sensitive and selective, cost-effective methods for the TB detection is of great importance. In recent years, scientists have employed a variety of new biosensing methods for the detection of TB, reducing the interval between sampling and obtaining the results of the tests (Lazcka et al. 2007). Incorporation of nanomaterials (NMs) and especially nanoparticles (NPs) with unique properties in biosensing design provides more simple, swift, sensitive and hybrid nano-biosensor platforms with synergetic properties and functions (Nosrati et al. 2017; Vigneshvar et al. 2016). Complexes of NPs such as gold nanoparticles (AuNPs) with different bio-recognition elements (antibodies, peptide arrays, polymers, and aptamers) provide many versatile strategies for improving the performance of detection systems (Perumal and Hashim 2014). Aptamers have recently been considered as highly promising biorecognition elements (Jalalian et al. 2018; Ko et al. 2013). Considering all reasoning mentioned above, we review here the recent advances in the field of nano-based aptasensors developed for *Mtb* detection. This review also presents some data on advantages of modified aptamer (SOMAmer)-based biosensors for *Mtb* diagnostics.

2. Aptasensors

Aptamers are small short single-stranded molecules of DNA or RNA creating a unique folding that specifically bind to the target molecule (including small substances, proteins and even whole cells) with significant specificity and affinity (Alibolandi et al. 2015; Mokhtarzadeh et al. 2015; Taghdisi et al. 2015). Aptamers with high affinity for a desired target are produced during a repetitive *in vitro* SELEX (systematic evolution of ligands by exponential enrichment) process (Chai et al. 2011; Szeitner et al. 2014). The ASSURED (**a**ffordable, **s**ensitive, **s**pecific, **u**ser-friendly, **r**apid and **r**obust, **e**quipment-free and **d**eliverable to end users) criteria, outlined by the WHO in terms of diagnostics for developing countries is mostly implemented by aptamer-based assays (Mabey et al. 2004). As a rule

of thumb, when compared with antibodies, aptamers exhibit unique properties such as lack of immunogenicity and toxicity, higher specificity and affinity, smaller molecular weights (for example, a DNA aptamer has an average molecular weight about 25 kDa), excellent thermal stability including storage at ambient temperature, ease of synthesis and modification and lower cost and time of production (Dehghani et al. 2018). Usually, production of a monoclonal antibody from an immunized rodent takes 43-47 days, while aptamers can be synthesized and purified within even one day. Furthermore, since aptamers are synthesized *in vitro*, the probability of virus contamination from the animal and other sources greatly wanes. Aptamer-based sensors (aptasensors) have received substantial attention within biosciences during the last few years (Ramezani et al. 2015; Shahdordizadeh et al. 2017).

2.1. Aptasensing of whole cells of *M. tuberculosis* and mycobacterial antigens

Although many serological approaches for the detection of *Mtb*-induced antibodies exist, during "a window of time", the mycobacterial antigens exist in the serum samples but host antibodies have not been produced. Therefore, evaluation of serum sample content during this period may cause a false negative response. In addition, current commercial or experimental serological approaches, cannot distinguish patients with active TB from BCG-vaccinated or latently infected individuals (Raja et al. 2008). Along these lines, the whole-bacterial selection by aptamers is a simple, straight forward and reproducible approach that can be carried out without prior knowledge of target molecules. In a study involving the use of aptamers against the whole-cells of *M. tuberculosis* (H37Rv) for therapeutic applications, the NK2 aptamer was found to express inhibitory effects on the *in vitro* adhesion/invasion of H37Rv strain to macrophages and to stimulate intracellular IFN-γ production in CD_4^+ T-cells. In this study, the aptamer NK2 was shown to prolong the survival of mice, lower the bacilli numbers in mice tissue, and decrease the pathological changes in the animal (Chen et al. 2007). It was further found to constrain *Mtb* (H37Rv) invasion of macrophage and to provoke IFN-c, IL-15 and IL-17 secretion by macrophages as notable serological cytokines (Chen et al. 2012).

In another study, the 10th pool of aptamers was found to have the strongest inhibitory effect against H37Rv adhesion/invasion to $CD8^+$ T-cells *in vitro* (Chen et al. 2013). While determining aptamer sequences that can affect the specific binding, Mozioglu and co-workers clearly demonstrated that highly specific aptamers contained "G-repeats" on the 3′ end of their structures and were enriched in "TGGGG," "GTGG," and "CTGG" motifs (Mozioglu et al. 2016). Several studies have demonstrated the potential of aptamers in targeting essential proteins (M Dupont et al. 2011) and *Mycobacterium tuberculosis* components like acetohydroxy acid synthase (AHAS) (Baig et al. 2015), mannose-capped lipoarabinomannan (ManLAM) (Pan et al. 2014) and polyphosphate kinase 2 (Shum et al. 2011). ManLAM, the major surface lipoglycan of *M. tuberculosis,* is an immunosuppressive epitope of *M. tb*. An aptamer (ZXL1) that specifically bound to ManLAM from the virulent *Mtb* strain H37Rv was generated. Injection of ZXL1 competed with the mannose receptor for binding to ManLAM and *Mtb* H37Rv and significantly inhibited the ManLAM-induced immunosuppression and reduced the progression of *Mtb* H37Rv infections and bacterial loads in lungs of mice and rhesus monkeys (Pan et al. 2014). Therefore, the aptamer ZXL1 can be used as a new antimycobacterial agent and tuberculosis vaccine immune adjuvant (Fig. 1).

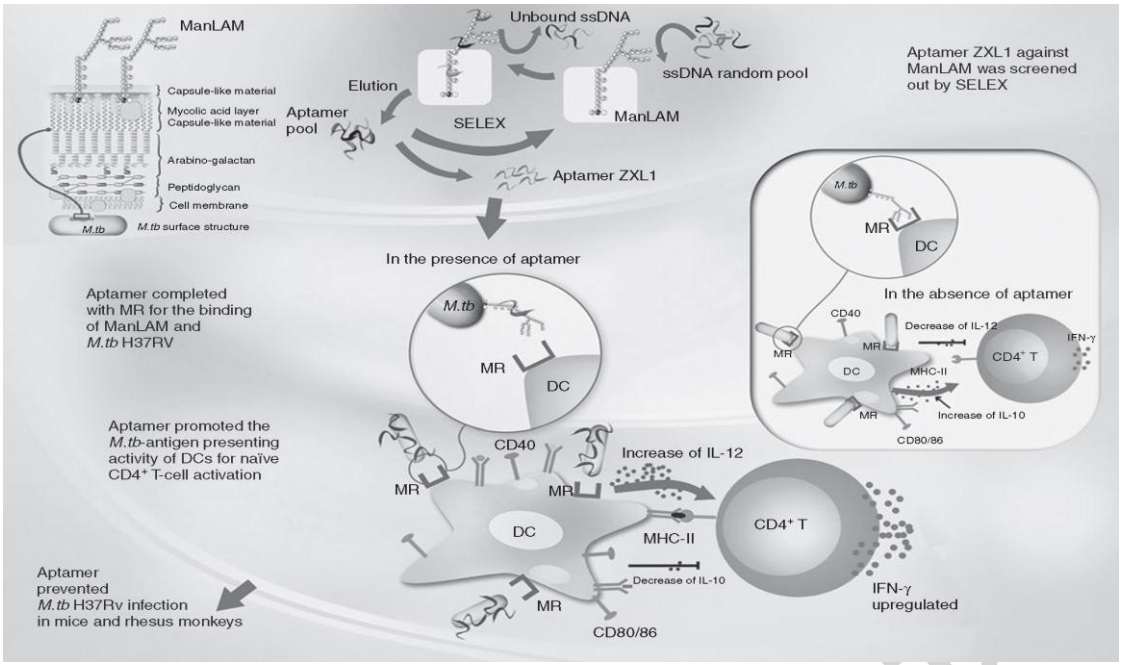

Fig. 1. Schematic illustration of aptamer ZXL1-mediated prevention of *Mtb* H37Rv infection through blocking of mannose-capped lipoarabinomannan (ManLAM)-induced immunosuppression. Reprint from (Pan et al. 2014) under a creative commons license.

Naturally, host immune system responses to the infection by antibody secretion, can be used as diagnostic and prognostic indicators of pathogen presence at various stages of infection. However, the recombinant proteins obtained from different batches processing and/or contaminating factors derived from the cloning vectors make difference in antigenic properties, resulting in variability in assay accuracy (Baassi et al. 2009; Silva et al. 2008; Tang et al. 2014). For this reason, Zhu *et al*. (Zhu et al. 2012) developed a sandwich ELISA method based on biotinylated aptamers against anti-MPT64 antibodies (antibodies secreted only by the MTB complex induction) with the aim of diagnosis of pulmonary tuberculosis. The minimum LOD of the developed ELISA method was 2.5 mg L^{-1} with a linear range varying from 10-800 mg L^{-1}. In addition, high specificity (99.4 %) and sensitivity (66.4 %) were detected, suggesting this method would be of potential clinical value. MPT64 is a 23 kDa secreted protein encoded by *Mtb* and virulent *M. bovis* strains and is distinctively absent from most of the BCG strains (Oettinger and Andersen 1994; Silva et al. 2008). The investigation of sequences responsible for specificity to MPT64, led Sypabekova and collaborators to observe a relationship between the length of TCCAGT sequence repeats and aptamer specificity for MPT64 (Fig. 2A) (Sypabekova et al. 2017). The detection limit can be improved when integration of inherently conducting polymers (ICPs) or other electroactive nanomaterials is considered. For this purpose, Bai and his group synthesized fullerene-doped polyaniline (C_{60}-Pan) nanohybrid constructs decorated with MPT64-specific aptamers (Fig. 2B) to obtain an ultrasensitive electrochemical aptasensor with LOD of 20 fg mL^{-1} (Bai et al. 2017). Mixing ICPs with other nanomaterials (such as graphene, fullerene, carbon nanotubes, and silicon-based nanomaterials) provides a high surface area due to the highly porous structure of ICPs, numerous active groups for conjugation of biomolecules and excellent electrical properties. Recently, a DNA aptasensor has been developed using immobilizing MPT64-specific aptamers to conducting polymer poly (3,4-ethylenedioxythiophene) (PEDOT) doped with carbon nanotubes (Fig. 2C) (Thakur et al. 2017). PEDOT has some noticeable properties among the other ICPs (such as polyaniline (PANI), polypyrrole (PPy), polyacetylene (PA), polythiophene (PT), poly (p-phenylene sulfide) and poly (p-phenylene vinylene)) such as prominent conductivity, intrinsic biocompatibility and higher thermal, chemical and electrical stability (Mandal et al. 2014; Rad and

Valipour 2015). This sensor showed a unique detection limit of 0.5 ± 0.2 fg mL^{-1} only within 15 minutes and up to seven times reusability. Although an automated culture of *Mtb* yields a LOD of 10 cfu mL^{-1}, equivalent to 0.1 fg mL^{-1} of MPT64, which is lower than the LOD of the aforementioned aptasensor, the culture-based method takes six weeks for a positive result.

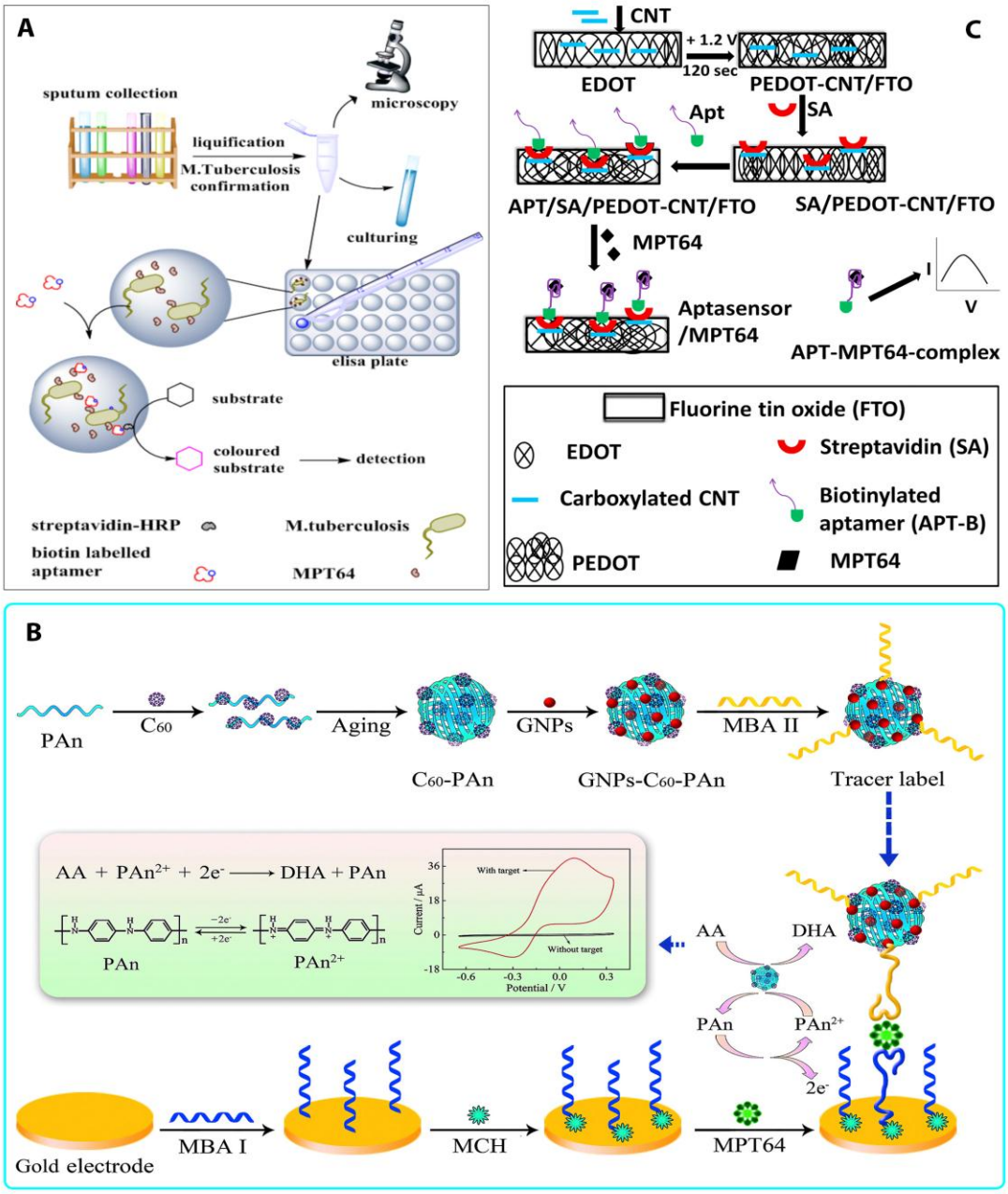

Fig. 2. Examples of aptasensors developed for MPT64 detection (A) Detection of MPT64 with an aptamer based sandwich enzyme linked oligonucleotide assay (ELONA) techniques, Reprint from (Sypabekova et al. 2017) under a creative commons license; (B) Detection of MPT64 with a DNA aptasensor immobilized on PEDOT-CNT/FTO, reprinted with permission from (Thakur et al. 2017); (C) Detection of MPT64 with specific aptamers and fullerene-doped polyaniline (C_{60}-Pan) nanohybrid. reprinted with permission from (Bai et al. 2017).

Antibodies are classified as complex targets of the SELEX process and therefore, it is more likely that further aptamers with higher affinities (greater than one) will be produced against the target(s), with different binding orientation relevant to each other. With a little optimism, we can implement an

improved serological diagnostic platform for TB characterization by more than one aptamer that each of them binds to anti-MPT64 antibodies in different orientations. This is feasible via monitoring the dynamic evolution of aptamers against complex targets like antibodies. According to this framework, additionally evolved aptamers with different epitope affinity, can promote the formation of a sandwich platform, in which one aptamer can be used to capture the target molecule and another for its detection (Qin et al. 2014). To date, five sub-classes of type VII secretion systems (T7S) have been observed in mycobacteria and are classified as ESX-1 to ESX-5 secretion systems. It has been proved that proteins secreted by the ESX-1 secretion system (e.g. the well-characterized ESAT-6 and CFP-10 *Mtb* proteins) can modulate pathogenicity of the bacilli in the host (Pym et al. 2003). However, proteins involved in the ESX-3 secretion system of *Mtb*, like *EsxG*, have enormous homology to those of ESX-1 (Abdallah et al. 2007; Serafini et al. 2009). Considering the above concept, aptamers against members of each family can be utilized in the detection or future reduction in *Mtb*-immune system caused destructions. Based on this idea, the development of RNA aptamers against *EsxG*, an ESX-3 secretion system protein whose role in bacterial response to iron and zinc in varying environments is being studied (using SPR-based SELEX). Aptamers suitable for binding to *EsxG* were found to have a remarkable lower affinity for its homologues, *EsxA* (Ngubane et al. 2014).

Numerous investigations have reported the major role of the ESX-1 secretion system protein, 6 kDa early secretory antigenic target (ESAT-6), in induction of apoptosis as a crucial component in TB progression. Live attenuated strains like BCG and live-attenuated *phoP*-/DIM-deficient *M. tuberculosis* strain MTBVAC that lack functional *ESX-1* locus are unable to cause apoptosis and cell death (Aguilo et al. 2013; Aporta et al. 2012; Arbues et al. 2013; Choi et al. 2010; Derrick and Morris 2007; Pym et al. 2002). Accordingly, TB antigens have been applied for induction and investigation of the apoptosis process in a luciferase biosensor. A recombinant firefly luciferase designed to covalently incorporate through the N and C termini a linker peptide containing the caspase 3/7 cleavage sequence, DEVDG, was studied in this aspect. During apoptosis induction by *esxA* (ESAT-6), *esxT* and *esxL* mycobacterial proteins, caspase activation occurs and subsequently cleaves the inlayed domain. Before linker peptide removal, the luciferase is in an open state and its emission is limited while with caspase-mediated linearization of recombinant luciferase (toward closed state), a high emission occurs signaling apoptosis (Shi et al. 2014).

As previously mentioned, the CFP-10 protein, encoded by the Rv3874 (*esxB*) gene, and the ESAT-6 protein, encoded by the Rv3875 (*esxA*) gene, may interact with each other *in vitro* to form a tight 1:1 heterodimer (Renshaw et al. 2005; Renshaw et al. 2002). The CFP10-ESAT6 complex secreted only by *M. tuberculosis* in its early culture time, showed higher sensitivity for detection of *Mtb* existence compared to other single antigens produced by the bacilli (Philip et al. 2005; Renshaw et al. 2002). These two unique TB biomarkers are not present in many nontuberculous mycobacteria and in the *M. bovis* BCG vaccine strain (Alderson et al. 2000; Andersen 1994; Skjøt et al. 2000). In a study using the anti-CFP10-ESAT6 heterodimer aptamers, biotinylated aptamers were screened in an enzyme-linked oligonucleotide assay (ELONA) along suspicious sputum specimens. The experiment demonstrated that one out of the six selected aptamers (CSIR 2.11), showed sensitivity and specificity of 100% and 68.75% respectively using Youden's index and 35% and 95% using a rule-in cut-point, respectively (Rotherham et al. 2012). However, in another study by Tang *et al*. (Tang et al. 2014) involving the CE24 or CE15 aptamers incorporated in an aptamer-based ELONA a much higher specificity (94.1%) than the CSIR2.11 aptamer was observed using Youden's index (sensitivity and specificity of CE24: 100% and 94.1%; sensitivity and specificity of CE15: 89.6% and 94.1%, respectively). The efficiency of the aptamer-based ELONA and T-SPOT (Oxford Immunotec, Oxford, United Kingdom) tests within TB patients was further compared to "similar detection limits for both methods for TB diagnosis". Interestingly, one out of 15 health care personnel and five infant TB

patients were found to be TB positive with ELONA but produced a negative response with the T-SPOT method. The investigators attributed the negative response to immunodeficient patients as the T-SPOT assay measures the release of IFN-γ from T cells. Since the immune system and T cells of infants or immunodeficient persons, rather than adults, are not functional and cannot secrete adequate amounts of IFN-γ molecules, it was possible that the levels of IFN-γ were not adequate as compared to those of immunocompetent adults. However, the ELONA-based aptamer assay directly evaluates the mycobacterial antigens in individuals and does not depend on the host immune situation. Moreover, the aptamer-based ELONA method can discriminate active rather to inactive TB (Tang et al. 2016).

Aptamers not only bind to their target protein via their tertiary structure, but can also compete with a complementary nucleic acid to form double-stranded configurations. The binding force is much stronger for the target protein than those for a complementary nucleic acid are (Dittmer et al. 2004; Hamaguchi et al. 2001; Nutiu and Li 2003). Based on this affinity difference, an Au-IDE/CFP10-ESAT6 aptamer/DNA- AuNPs MSPQC probe was constructed by modifying CFP10-ESAT6 specific aptamers on the surface of an interdigital electrode through Au–thiol binding and subsequent hybridization of the complementary DNA-AuNPs with CFP10-ESAT6 specific aptamers. When the protein CFP10-ESAT6 was present in the solution, it would bind to the CFP10-ESAT6 aptamer and the complementary DNA-AuNPs would in turn detach from the electrode surface. Because of weak conductive properties of the CFP10-ESAT6, the electrical properties of interdigital electrode surface would undergo changes following detachment of the conductive material, namely the DNA-AuNPs. The measured frequency shift (Hz) in the typical response curve for 10^6 cfu mL^{-1} *Mtb* was 121 Hz while the oscillating frequency change resulted by other than *Mtb* bacteria did not exceed 15 Hz (He et al. 2016). The "corona shield effect" phenomenon usually appears when AuNPs-based aptasensors are used for detection of targets in complex samples like serum. The surface modification of AuNPs may be an alternative strategy to overcome this limitations (Dreaden et al. 2012). Table 1 summarizes the list of aptamers selected for various types of mycobacterial targets with different efficiencies.

Table 1. Aptamers used to target various components of *Mtb*.

Name of aptamer	target	Biosensing method or aim of use	Measured efficiency parameter	Linear range	Aptamer sequence	Reference
NK2	Whole *M. tuberculosis* (H37Rv) cell	ITC	Not reported	Not reported	Not reported completely	(Chen et al. 2007)
MPT64-A1	Anti-MPT64 antibody	ELISA	LOD: 2.5 mg L^{-1}	10 to 800 mg L^{-1}	5′ -GGGAGCTCAGAATAAACGCTCAAAAACGCTCA AGAGGCCCGGATCTTCGACATGAGGCCCGGATC-3′	(Zhu et al. 2012)
PAA1	Anti-MPT64 antibody	ELISA	K_d: 8.68 nmol L^{-1}	-	5′ -GGGAGCTCAGAATAAACGCTCAACGCATTTCGC AACACGACTTGGCCAACGTTCCTGGTTCGACATGAGG CCCGGATC-3′	(Qin et al. 2014)
NK2	Whole *M. tuberculosis* (H37Rv) cell	Flow cytometry	K_d: 31 ± 4	-	Not reported completely	(Chen et al. 2012)
Mtb36	Whole *M. tuberculosis* (H37Rv) cell	qPCR	K_d: 5.09±1.43 nM	-	5′ -TTGGTTGCTGAATCCCCTCGTCTTGGCTTTC TTTGTCGGG-3′	(Mozioglu et al. 2016)
M64CA	MPT64	biotinstreptavidin-horseradish peroxidase system	OD: 0.58 as cut-off	0.5 to 2 mg L^{-1}	5′ -GGGAGCTCAGAATAAACGCTCAATTCGGGA ATGATTATCAAATTTATGCCCTCTGATTTCGACATGA GGCCCGGATC--3′	(Qin et al. 2009)
-	MPT64	DPV	0.5 ± 0.2 fg mL^{-1}	1.0×10^7 fg m L^{-1} to 1.0×10^3 fg m L^{-1}	5'-GTACAAACGACGGCCAGTCCTTGGGATGATTCAA GCAAAGCCTCACGCCTACGGCTAAGTCATAGCTGTCT CTCCTG-3'	(Thakur et al. 2017)
MBA I	MPT64	DPV	20 fg mL^{-1}	0.02 to 1000 pg mL^{-1}	5'-SH-$(CH_2)_6$-TGGGAGCTGATGTCGCATGGGTTTTGATC ACATGA-3'	(Bai et al. 2017)
-	CFP-10.ESAT-6 heterodimer	MSPQC	Frequency shift: 121 Hz	-	5′ -GGGAGCTCAGAATAAACGCTCAAACGTATTCC ATAGTGCTTTATTACTCGCACTTGTGTTCGACATGAG GCCCGGATC-C6-3′	(He et al. 2016)

CE15 and CE24	ESAT6 and CFP10, respectively	ELONA	K_d: 1.6 ×10^{-7} M & 3.75 × 10^{-7} M	-	Not reported completely	(Tang et al. 2014)
CSIR 2.11	CFP-10.ESAT-6 heterodimer	ELONA	K_d: 861.07 nM	-	Not reported completely	(Rotherham et al. 2012)
Mtb-Apt1 and Mtb-Apt6	AHAS	To inhibitory purposes	IC_{50}: 28.9 ± 0.002 & 22.3 ± 0.001, respectively	-	Mtb-Apt1: 5′ - CGAGTGAGGGCGAGGCGCGCTCCTGCCGGT-3′ Mtb-Apt6: 5′ - CGGCCAGGGGACGAGCGCGCCCTGATCGTG-3′	(Baig et al. 2015)
T9	ManLAM	ELONA	K_d: 668 ± 159 nmol L^{-1}	-	Not reported completely	(Tang et al. 2016)
ZXL1	ManLAM	ELONA	K_d: 436.3 ± 37.84 nmol L^{-1}	-	5′ -GCGGAATTCTAATACGACTCACTATAGGGAACA GTCCGAGCCNGGCGCCATAGCGACGGGGCCATTCCA AGAAGGGTCAATGCGTCATA-3′	(Pan et al. 2014)
-	ManLAM	enzyme linked aptamer assay	LOQ for direct assay: 104CFU/mL LOQ for indirect assay: 105CFU/mL	-	For direct dot-blot: 5′ - GGCGCCATAGCGACGGGGCCATTCCAAGAA-biotin-3′ For direct dot-blot: 5′ - GCGGAATTCTAATACGACTCACTATAGGGAACAGTCC GAGCCGGCGCCATAGCGACGGGGCCATTCCAAGAA-GGGTCAATGCGTCATA-3′	(Li et al. 2018)

ITC: Isothermal titration calorimetry, **LOD:** Limit of Detection, **MSPQC:** Multiple channel series piezoelectric quartz crystal, **AHAS:** Acetohydroxyacid synthase, **qPCR:** quantitative PCR, **ELONA:** enzyme-linked oligonucleotide assay, K_d**:** dissociation constant.

2.2. Aptasensing interferon gamma

The motivation to develop new technologies for cytokine detection especially during exposure of the immune system to mycobacterial antigens is becoming increasingly stronger. IFN- γ production occurs mainly by macrophages and T helper cells ($CD4^+$) upon stimulation with antigens, which is used to determine prior exposure to infectious diseases. From a clinical perspective, IFN-γ release by T cells is an evidence for the diagnosis of latent tuberculosis while in the laboratory it is used to verify the presence of disease-derived immune T cells. The main role of IFN-γ is to modulate cellular immune response to viral and mycobacterial infections (Chesler and Reiss 2002; Flynn et al. 1993; Harari et al. 2004).

Today, antibody-based immunoassays like ELISA, ELISpot and Western blot are considered as gold standards in detecting cytokines as pathogen-stimulated factors (Mäkitalo et al. 2002). Although these immunoassays are specific and sensitive in characterizing cytokines, they are often expensive, their application is cumbersome, time-consuming, involving multiple washing steps and requiring large sample volumes for analysis (Liu et al. 2012). Specific aptamers against, or involved in IFN-γ-associated routes have been reported by various research groups (Balasubramanian et al. 1998; Fedoseyeva et al. 1994; Lee et al. 1996; Ramanathan et al. 1994; Tam et al. 1994). Detection of IFN-γ by a Methylene Blue (MB) redox tagged DNA hairpin aptamer-based biosensor was recently described by Liu *et al*. (Liu et al. 2010). A micro device for detecting local interferon gamma release from anti-$CD4^+$ antibodies-captured human leukocytes in real-time (upon injection of blood), utilizing the same MB redox label attached DNA hairpin aptamer-modified electrodes integrated with microfluidics was developed with excellent sensitivity for IFN-γ (limit of detection < 60 pM). Applying inexpensive and portable micro-chips for fabrication of this aptasensor, probably can make it a useful device for the point-of-care (POC) diagnosis. Interestingly the device did not require further washing or labeling steps. The observed change (decrease) in electrical signal was attributed to unfolding of the structure of the hairpin and displacement of the redox marker from the electrode (Liu et al. 2011). This design improved the sensitivity of detection. However, it has some drawbacks such as difficulty for its mass production when using glass as a substrate and almost impossible integration of the electrical circuit beneath the glass substrate to construct multiple electrode systems. To address this, a typical photolithography process fitted for large-scale production of microelectronic tools was employed to fabricate the device on a silicon substrate with low cost and a highly sensitive array of

gold electrodes. Silicon has the advantage to incorporate active electrical parts on chips in order to improve operation speed, system reliability and equipment density (Chen et al. 2014). Recently, A triple-helix molecular switch (THMS)-based electrochemical aptasensor for IFN-γ using a gold electrode and MB as a redox probe was described by Abnous et al. (2017). In the presence of IFN-γ, the structure of the THMS is disassembled and MB can only bind to the signal transduction probe (STP) on the surface of the gold electrode, leading to a weak peak current. However, the strong current was observed in the absence of IFN-γ due to the fact that MB could not interact with the THMS (Fig. 3A). Under optimized conditions, the assay has a 3 pg mL^{-1} detection limit and a linear range that extends from 10 to 1500 pg mL^{-1} (Abnous et al. 2017).

Aptamer-assisted IFN-γ sensing has also been monitored by fluorescence resonance energy transfer (FRET)-based detection (Tuleuova et al. 2010) and SPR (Chang et al. 2012; Chuang et al. 2014). The impedance spectrum acquired for RNA and DNA aptamers indicated that the RNA aptamer probe could bind to IFN-γ 10 times more than the DNA aptamers. LOD for IFN-γ in a FBS (Fetal Bovine Serum) solution with the DNA aptamer was 10 pM with a significant increase in R_{ct}. However, there was a slight decrease in R_{ct} values for the RNA aptamer probe in the FBS buffer which may be attributed to tiny amounts of RNase enzymes in FBS test solution. FBS solutions have similar components to pleural fluid, hence investigation for IFN-γ in FBS closely relates to a study in physiological body fluids (Min et al. 2008). Recently, a fluorescent aptasensor was designed for IFN-γ detection could be detected concentration as low as 1 pg mL^{-1} IFN-γ with a LR between 10 pg mL^{-1} and 4 ng mL^{-1}, under optimal conditions. In this platform, without introduction of IFN-γ, the hairpin structure of oligonucleotide is preserved on the surface of SNPs-Streptavidin and SSB could not bind to poly-T sequence. So, fluorescent CuNPs were formed on the surface of SNPs-Streptavidin, leading to a strong fluorescence signal. In the presence of IFN-γ, the hairpin structure of oligonucleotide is destructed and SSB could bind to the released poly-T strand (Fig. 3B). Thus, the fluorescent CuNPs were not formed on the surface of SNPs-Streptavidin, resulting in a weak fluorescence intensity (Taghdisi et al. 2017). Despite studies demonstrating that fluorescent biosensors are useful for IFN-γ detection, the intrinsic fluorescence of some proteins in serum may interfere with the outcome of the tests and thus the fluorophores with time-resolved fluorescence properties should be used (Alexiev et al. 2017). Some nanomaterials such as AuNPs and graphene oxide (GO) have super-quencher properties and can be incorporated into fluorescence aptasensors to increase sensitivity and reduce background signals (Li et al. 2015a).

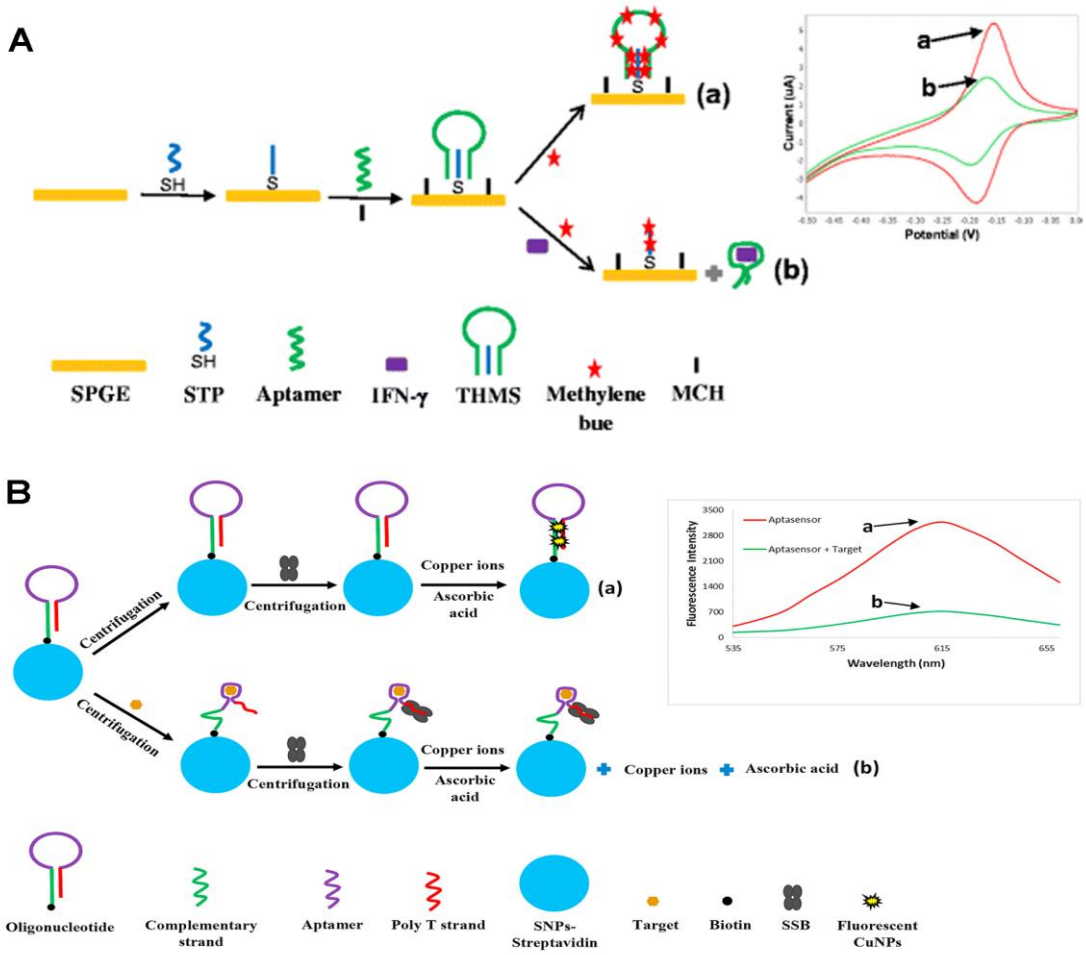

Fig. 3. Schematic illustrations of IFN-γ detection by the THMS-based electrochemical aptasensor (A) and using the fluorescent aptasensor (B). Reprinted with permission from (Abnous et al. 2017) and (Taghdisi et al. 2017).

During the last decade, graphene has attracted enormous interest also in the development of biosensing technologies. Graphene has unique ambipolar characteristics and a large surface area that has extraordinary electronic transport properties making it a suitable candidate for biomolecular sensing (Li et al. 2009; Stankovich et al. 2006). Yan *et al*. reported a label-free and reliable sensitive electrochemical aptasensor for IFN-γ based on controlled assembly of graphene and design strategies based on enzyme cleavage-assisted target recycling amplification (Fig. 4A). In their work, a gold electrode surface was modified by 16-mercaptohexadecanoic acid (MHA). When the medium was free of IFN-γ, graphene could not assemble on the surface of the electrode because of the strong adsorption of IFN-γ binding aptamers on the graphene via π–π interactions causing a halt on electronic transmission. However, in the presence of IFN-γ and DNase I, desorption of the interacting aptamers from graphene and subsequent aptamer digestion occurred, resulting in the release of the interferon. The released IFN-γ could in turn interact with other graphene-adsorbed aptamers causing further release from the graphene. Finally, the aptamer-cleared graphene could successfully assemble onto the MHA modified electrode via hydrophobic and π-π interactions, mediating the transfer of electrons between the electrode and graphene. The calculated limit of detection for IFN-γ in the latter study was 0.065 pM (Yan et al. 2013). The detection of IFN-γ, was moreover implemented by using a graphene-based electrochemical biosensor where the graphene platform successfully replaced a common gold electrode (Farid et al. 2015). The establishment of multi-analyte aptasensors for the identification of different cytokines secreted by cells in which each specific aptamer for different

cytokines labels with diverse redox reporters is of importance. When such aptamers assemble as a random mixture on the surface of a common electrode, monitoring the shifting of the redox peak allows selective detection of different cytokines simultaneously (Liu et al. 2015b). Recently, a new electrochemical aptasensor for IFN-γ assay, based on a graphene/AuNPs modified electrode coupled with a dual enzyme-assisted signal amplification was developed with an ultra-low detection limit of 2 pM while exhibited a wide linear range from 5 pM–5 nM (Fig. 4B). In the presence of IFN-γ, the hybridization of aptamer/IFN-γ led to the release of aptamers from the electrode surface. Simultaneously, aptamers were digested with RecJf exonuclease and IFN-γ was available for target recycling as capture probes and hybridized with linker probes and biotin-labeled reporter probes. As a result, cascade duplex DNA polymers were produced on the electrode surface, providing lots of binding sites for streptavidin–alkaline phosphatase to generate strong enzyme-catalyzed signals (Yin et al. 2017). It should be noted that when graphene-based aptasensors are used for the detection of targets in complex samples like serum, the sensitivity of the sensing platform may be altered by the interaction of serum proteins with grapheme. Pre-treatment of GO with molecules like albumin or PEG may be used to solve this issue (Chung et al. 2013).

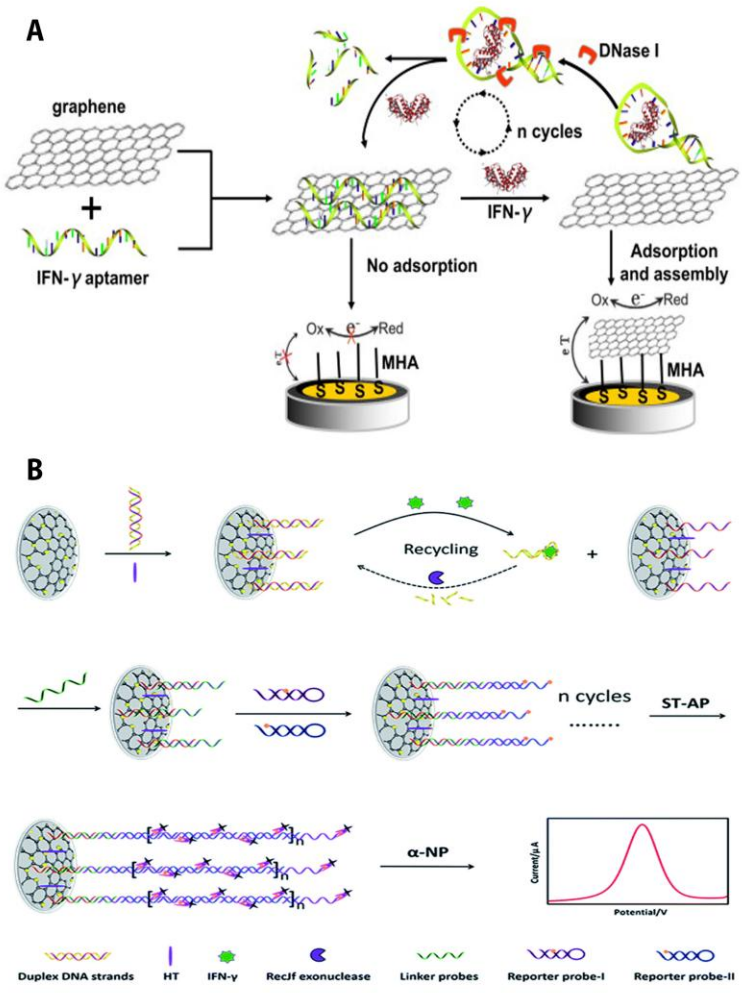

Fig. 4. The schematic representation of the electrochemical aptasensor for the IFN-γ detection (A) based on graphene controlled assembly and nuclease cleavage-assisted target recycling amplification strategy, reprinted with permission from published paper (Yan et al. 2013); (B) based on a graphene/AuNPs modified electrode coupled with a dual enzyme-assisted signal amplification strategy. Reprint from (Yin et al. 2017) under a creative commons license (CC BY-NC 3.0).

Various signal amplification strategies have been employed in the design of aptasensors for ultrasensitive bioassays. Furthermore, numerous DNA amplification technologies, such as RCA (Zhao et al. 2008), hybridization chain reaction (HCR) (Dirks and Pierce 2004), and branched DNA technology (Tsongalis 2006) have been explored as alternative strategies for sensitive biosensor assays. Zhao *et al*. developed a novel electrochemical aptasensor based on HCR for the detection of IFN-γ (Zhao et al. 2012). The HCR amplification method is an isothermal amplification approach involving two co-existing species of DNA hairpins in the solution until an initiator strand is entered. By opening the first species of DNA hairpin, they lose their secondary structure, causing subsequent stimulation of the opening of the hairpins of the second species. Finally, the initiator strand triggers cascades of hybridization that produce nicked double helices similar to alternating copolymers. In HCR studies, target DNA often triggers HCR amplification as initiator while fluorophore-labeled DNA hairpins open one after another, offering a significant and sensitive signal for target DNA detection. In the aptasensor designed by Zhao and coworkers, the recognition probes possessed the sequence of IFN-γ aptamer, which eventually would bind to IFN-γ. In this manner, unbound recognition probes, were captured on the electrode as initiators triggering the HCR amplification (Dirks and Pierce 2004). The DNA hairpins bio-H1 and bio-H2 were opened by the recognizing probe, and bound to each other on the electrode. In this experiment, biotin was applied as a tracer in the hairpins and streptavidin–alkaline phosphatase (SA–ALP) as a reporter complex. When SA–ALP converts its electro-inactive substrate 1-naphthyl phosphate into the electro active derivative 1-naphthol, it yields an elevated electrochemical signal by differential pulse voltammetry (DPV).

A novel electrochemical aptasensor was introduced by Liu *et al.* for the IFN-γ detection based on the exonuclease-catalyzed target recycling and signal amplification cascade by the TdT-mediated framework. In this aptasensor, an AuNP-graphene electrode was immobilized using previously hybridized capture probes with complementary IFN-γ aptamer. The presence of IFN-γ led to the formation of a complex between IFN-γ and the hybridized aptamer. Through emancipation of the aptamer, the exonuclease selectively digested the aptamer, and consequently IFN-γ was released for the recycling of the target. Single-stranded capture probes were hybridized with a signal probe labeled with Au−Fe_3O_4. Terminal deoxynucleotidyl transferase (TdT) catalyzed the labeled signal probe sequences at the 3′-OH group to construct a long ssDNA structure. Finally, the electron mediator hexaammine ruthenium (III) chloride ($[Ru(NH_3)_6]^{3+}$) adsorbed to DNA via electrostatic interactions resulted in creating a significant electrochemical signal for quantitatively measuring IFN-γ levels (Fig. 5) (Liu et al. 2015a). Table 2 summarizes the different types of aptamers used in biosensing or inhibitory approaches on IFN-γ.

The aptamer-based methods used for the detection of TB, currently reported in literature, only worked on laboratory scale and we did not find reports about any direct assays on patient samples and, to the best of our knowledge, no reports on methods being employed in clinic in recent time.

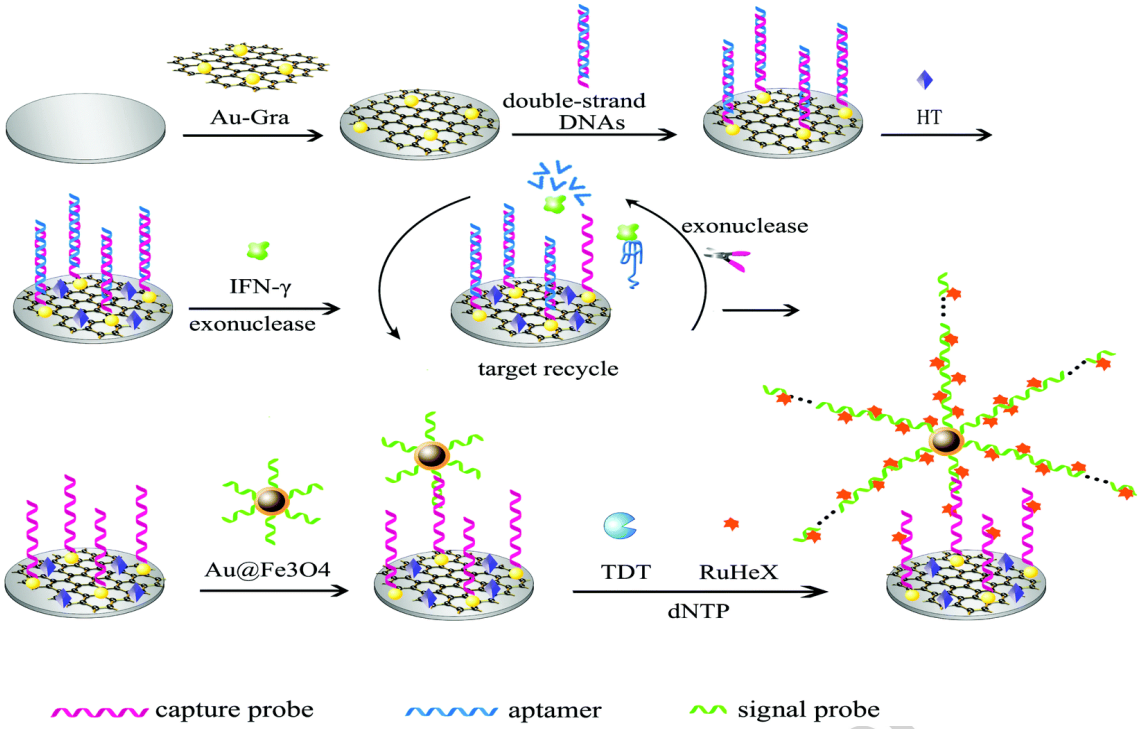

Fig. 5. Schematic illustration of the stepwise aptasensor fabrication based on exonuclease-catalyzed target recycling and surface-initiated enzymatic polymerization for amplification. Reprinted with permission from (Liu et al. 2015a)

Table 2. Aptamers used to target IFN-γ.

Biosensing method	Aptamer type	Measured efficiency parameter	Linear range	Sequence	Reference
Electrochemical	DNA & RNA, respectively	LOD: 1 Pm & 100 fM, respectively*	–	5′-HS-$(CH_2)_{12}$-GGGGTTGGTTGTGTTGGG TGTTGTGT-3′ & 5′-HS-$(CH_2)_{12}$-GGGAGGAC GAUGCGGA CA CCGUUAAUCUGAGGCCCUGUCCUAUUCCUU CACGCCUCAGA-3′, respectively	(Min et al. 2008)
	DNA	LOD: 0.06 nM	Extending to 10 nM	5′-NH_2-C6-GGGGTTGGTTGTGTT GGGTGTTGTGTCCAACCCC-C3-SH-3′	(Liu et al. 2010)
		LOD: 58 pM	58 pM to 9.3 nM	5′-NH_2-C6GGGGTTGGTTGTGTTG GGTGTTGTGTCCAACCCCC3-SH-3′	(Liu et al. 2011)
		LOD: 0.003 ng mL^{-1}	0.01 to 50 ng mL^{-1}	5′-AAAGGGGTTGGTTGTGT TGGGTGTTGTGTCCAACCCC-3′	(Liu et al. 2015a)
		LOD: 0.065 pM	0.1 to 0.7 pM	5′-GGGGTTGGTTGTGTTGGGTGTTGTGT-3′	(Yan et al. 2013)
		LOD: 1.3 ng m L^{-1}	1 to 500 ng mL^{-1}	5′-NH_2–C6–GGGGTTGGTTGTGTTGGG TGTTGTGTCCAACCC- CC3–SH-3′	(Chen et al. 2014)
		LOD: 6.35 ng mL^{-1}	9 to 88ng mL^{-1}	5′-NH_2-C6-GGGGTTGGTTGTGTTGGG TGTTGTGTCCAACCCC-C3-SH-3′	(Liu et al. 2015b)
		LOD: 83 pM	–	5′-/Pyrene/GGGGTTGGTTGT GTTGGGTGTTGTGT-3′§	(Farid et al. 2015)
		–	–	5′-NH_2-C6-GGGGTTGGTTGTGTTGGGTGTT GTGTCCAACCCCC3-SH-3′	(Liu et al. 2012)
		3 pg mL^{-1}	10 to 1500 pg mL^{-1}	5′-CTCTCTGGGGTTGGTTGTGTTGGG TGTTGTGTTCTCTC-3′	(Abnous et al. 2017)
		LOD: 33 pM	0.3 to 333 nM	5′-GGGGTTGGTTGTGTTGGGTGTTGTGT-3′§	(Chang et al. 2012)
SPR	DNA	K_d: 3.44 nM	–	5′-GGGGTTGGTTGTGTTGGGTGTTGTGT-Biotin-3′	(Tuleuova et al. 2010)
		LOD: 10 pM	0.01 to 100 nM	5′-GGGGTTGGTTGTGTTGGGTGTTGTGT-3′′	(Chuang et

					al. 2014)
Fluorescens	DNA	1 pg mL^{-1}	10 pg mL^{-1} and 4 ng mL^{-1}	5′-Biotin- CCAAAAAAAAAAAAAAAAAAAAA GGGGTTGGTTGTGTTGGGTGTTGTGTATTTTTTTTTT TTTTTTTTTTT-3'	(Taghdisi et al. 2017)
For inhibitory purposes	DNA	beginning from 1 μM	–	5′-GGGGTTGGTTGTGTTGGGTGTTGT GT-RNH_2-3′	(Lee et al. 1996)

* Values in sodium phosphate buffer, IFN-γ detected to 10 pM by the DNA aptamer in fetal bovine serum.
† Following streptavidin-binding sequence has not been shown.
§ Aptamer with a 5' pyrene modification.

2.3. SOMAmer-based detection of tuberculosis biomarkers

Slow off-rate modified aptamers (SOMAmers) are a new class of synthetic ssDNA aptamers that contain pyrimidine residues hydrophobic modified at their 5-position (Candia et al. 2017; De Groote et al. 2013). Compared to standard RNA or DNA aptamers, the affinity of these reagents is magnitudes higher due to improvement of binding properties and the incorporation of modified nucleotides (Nahid et al. 2014). In addition, low molecular weight, high multiplexing capabilities, low cross-reactivity, chemical stability are some advantages of SOMAmers compared to antibodies (De Groote et al. 2013). SOMAmers as a new generation of protein-capture modalities are the basis of the SOMAscan assay (Rohloff et al. 2014; Vaught et al. 2010). SOMAscan is a highly multiplexed, aptamer-based proteomic technology optimized for protein biomarker discovery, which is made possible by the synchronous measurement of a wide-ranging of protein targets (Candia et al. 2017; Gold et al. 2010; Nahid et al. 2014). SOMAscan is considered as a rapid, accurate, quantitative and non-culture based test that could be used for point-of-care diagnosis of biomarkers of TB in serum, plasma, or other biological matrices in a small (<100 ml) sample volume.

The first large-scale SOMAscan employing modified DNA aptamers for study of active TB was reported by De Groote *et al.* (De Groote et al. 2013). They assayed the changes in proteins from paired serum samples at enrollment and after 8 weeks of TB treatment from 39 patients with pulmonary TB. They identified multiple proteins that exhibited significant expression differences during the intensive phase of TB therapy. The top proteins that changed between baseline and 8 weeks of therapy were TSP4, TIMP-2, SEPR, MRC-2, antithrombin III, SAA, CRP, NPS-PLA2, LEAP-1, and LBP (De Groote et al. 2013). SOMAscan was applied in the same study to analyze serum samples collected at baseline and after 8 weeks of treatment from 39 patients with pulmonary TB. Serum proteins involved in the coagulation cascade, neutrophil activity, immunity, inflammation, and tissue remodeling associated with TB treatment response were identified. Coagulation factor V, SAA, XPNPEP1, PSME1, IL-11 Ra, HSP70, galectin-8, a2-antiplasmin, ECM1, YES, IGFBP-1, CATZ, BGN, LYNB, and IL-7 showed the most significant difference in expression after 8-week (Nahid et al. 2014). De Groote *et al.* designed a SOMAscan analysis to identify biomarkers of LTBI. They showed that the proteins in the stimulated supernatants that distinguished LTBI from controls included IL-2, monocyte chemotactic protein 2 (MCP-2), interferon gamma inducible protein-10 (IP-10), IFN-γ, tumor necrosis factor superfamily member 14 (TNFSF14), monokine induced by gamma interferon (MIG), and granzyme B ($P < 0.00001$) (De Groote et al. 2017a). Recently, the potential of SOMAmer reagents with sub nanomolar affinities for *M. tuberculosis* proteins (antigens 85A, 85B, 85C, GroES, GroEL2, DnaK, CFP10, KAD, CFP2, RplL, and Tpx) for the diagnosis of TB (Russell et al. 2017) and active pulmonary TB (De Groote et al. 2017b) was revealed.

3. Conclusions and future perspectives

Given that in countries with high rates of TB cases, access to cheaper reliable diagnostics is one of the most important goals in controlling the disease, biosensors can play a critical role. This review focused on a variety of aptasensors designed to detect TB with a detection sensitivity in the

femtomolar range for pathogen-raised biomolecules. Analysis of the works published during the last two decades revealed that incorporation of nanomaterial and aptamers (Barroso et al. 2018; Bernacka-Wojcik et al. 2013) provides great prospects in the fabrication of new TB biosensing platforms with very high detection speed, sensitivity and accuracy. Considering the importance of diagnosis in the latent phase, there is still a need for developing inexpensive devices with lower detection limits for clinical and screening purposes. In this review we summarized efficient SOMAmer-based assays using low sample volumes as compared to the routine aptamer-based methods. However, it seems that many of the platforms described here are significantly more expensive than applicable in developing countries so, planning simpler designs such as deoxyribozyme (Bengtson et al. 2017) or molecular beacons ("sensor with tiered output for dual targets": STODT) (Huang et al. 2017) based TB DNA sensors is crucial for multiplex analysis of MDR- and XDR- TB. We anticipate that in the near future, simple portable diagnostic tools capable of multi analyte detection meeting the needs of high-prevalence areas will evolve. For example, in a recently published work, low-price and high efficient direct (for multi-sample quantification) and indirect (for strain identification) dot-blot assays were developed using a ManLAM specific aptamer for LTBI screening and an android app for processing and sharing of images taken by a simple smartphone camera in order to comply with portability requirements (Li et al. 2018). With these in mind, application of biosensors from bench to clinical diagnosis can pave the route to global objectives of TB control.

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Conflict of interests

The authors declare no conflict of interests.

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Highlights

- Tuberculosis, is a major global health problem caused by the bacterium *Mycobacterium tuberculosis* (*Mtb*) complex.
- Interferon-gamma (IFN-γ) is a crucial biological marker in early diagnosis of tuberculosis.
- This review mainly focuses on the recently developed nano-based aptasensors for the whole cell *Mtb*, mycobacterial proteins and IFN-γ detection.
- This review covers the prominent roles of nanomaterials in the development of tuberculosis aptasensors.
- The advantages of modified aptamer (SOMAmer)-based biosensors for *Mtb* detection are discussed briefly.