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19

# The Use of Phages and Aptamers as Alternatives to Antibodies in Medical and Food Diagnostics

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## 1. Introduction

In the post-genomic and proteomic era, there is a better understanding of important physiological components such as DNA, RNA, proteins and small biological molecules, all of which have proven to be the mediums of disease progression. Identification and investigation of disease-specific biomarkers in the initial stage of a disease can greatly increase accuracy in diagnosis, treatment and even prevention. This approach offers great potential to significantly reduce disease-related mortality rates. There is an increasing need in the medical field for rapid, cheap and reliable diagnostic systems in order to detect all the well-known and recently identified biomarkers for different diseases. This identification is not a trivial exercise because these disease biomarkers are present in minute quantities in physiological conditions such as the bloodstream or body fluids, which are often contaminated with many other compounds that can hinder detection. Apart from biomarker diagnosis, another area of concern for human health has been food contamination. Trading of contaminated food between countries and high population mobility increases the potential for outbreaks and health risks posed by microbial pathogens and toxins in food. Food safety has become a global health goal. Periodic toxin and microbiological analyses of food samples are important to diagnose and prevent problems related to health and food safety. However, food-borne pathogens are mostly present in very low numbers among various other microorganisms, making their detection difficult. To be able to detect these disease carriers and biomarkers in their natural conditions, highly sensitive as well as specific recognition elements are required. It is necessary to develop detection techniques that are reliable, fast, easy, sensitive, selective, cost-effective and also suitable for real time, *in situ* monitoring. Such techniques to detect pathogens and biomarkers would not only

improve clinical success rates but also offer a great commercial advantage to the medical field and the food industry.

Conventional techniques for the detection of disease biomarkers, pathogens and toxins are immunology-based methods, polymerase chain reaction (PCR) based methods and culture and colony counting methods. Though these standard detection methods are sensitive, they lag behind in terms of detection time, taking from several hours to days to yield a response (Velusamy et al. 2010). Conventional analytical techniques like optical, chromatographic and electrochemical detection are faster, but have some limitations of equipment and cost. Furthermore, they are complicated and require highly trained personnel and extensive sample preparation. These constraints do not always allow frequent, real time or *in situ* monitoring of food or clinical samples. Thus, demands of high sensitivity, specificity, cost-effective, portable and rapid analyses have propelled the development of biosensors as novel diagnostic tools in the medical and food sectors.

Many diagnostic tools still rely on immunoassays and especially enzyme-linked immunosorbent assay (ELISA). Besides these classical tests, several phage and aptamer based sensors have also been proposed for a broad range of disease biomarkers or carriers such as antibodies, viruses, disease-related proteins, tumour cells, toxins and pathogens, among several others. The precise detection of these biomarkers or carriers before the onset of a disease can significantly revolutionise the medical field by providing cheap, fast, simple and easily produced diagnostic tests using phages and aptamers as their recognition elements. The wide range of assays that employ phages or aptamers to detect important clinical molecules, highlights the potential of these new receptors in clinical diagnostic tests and in food biosensors.

This chapter will define phages and aptamers and discuss their use as novel biorecognition elements in biosensors. We discuss two relevant cases in the field of biosensors: the use of diagnostics for clinical testing and the use of biosensors for food-related testing.

## 2. Biosensors

A biosensor is defined by the IUPAC as a self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is retained in direct spatial contact with a transduction element.

Biosensors can be categorised by the type of recognition element used, such as enzymatic, whole cell or affinity-based biosensors. Enzymes are proteins that catalyse specific chemical reactions and were the first molecular recognition elements to be included in biosensors. They are attractive sensor recognition elements because their use can convert the analyte into a sensor-detectable product, evaluate the modification of enzyme properties upon interaction with the analyte or detect an analyte that acts as the enzyme inhibitor or activator. In fact, the most widely studied and acclaimed sensor success story is that of the glucose biosensor, an enzymatic sensor (Newman & Turner 2005; Wang 2007). Whole-cell biosensors, the next classification of biosensors, often use a genetically engineered cell of either eukaryotic or prokaryotic origin, containing responsive transcriptional promoter elements as the biological component. These whole cell biosensors are used for the profiling of the toxicological effects of compounds and for risk assessment of chemical contaminants or of new compounds (Robbens et al. 2010). The third type of biosensors which are of higher relevance for diagnostics, are affinity-based biosensors. In these, the affinity-based receptor

molecule binds the analyte irreversibly and non-catalytically. The binding event between the target molecule and the bioreceptor, triggers a physicochemical change that can be measured by a transducer. In order to 'visualise' the binding event, the different transduction methods that are frequently used in these biosensors are optical, electrochemical or mass-based.

When the detection system requires a biomolecular recognition event, antibody-based detection methodologies are considered the standard assays in clinical analysis (Aizawa 1994; Stefan et al. 2000). These assays are well established and have been demonstrated to reach the required sensitivity and selectivity. However, the use of antibodies *in situ* detection methods and in the analysis of very complex samples could encounter some limitations mainly deriving from the nature and synthesis of these protein receptors. Antibodies are relatively cheap, but their production relies on the immune response of an animal. Besides the ethical problems related to the use of animals, it is also difficult to generate antibodies for toxic compounds or small compounds that cannot elicit an immune response. In order to avoid some of these drawbacks, recent advances in biotechnology, nanotechnology and surface chemistry offer the possibility of developing other novel, affinity-based recognition molecules that have been explored as alternatives to the traditionally used antibodies. This is a domain in which phages and aptamers can play a successful role. They have emerged as viable options thanks to their high selectivity and affinity towards their targets, comparable to that of antibodies. The high affinity and hence high sensitivity, high specificity, robustness, animal-friendly production and ease of modification are some of the defining properties that make the use of aptamers and phages advantageous in diagnostic and biosensing tools (Van Dorst et al. 2010b).

## 3. Phages

Phages are viruses that use their host bacterial cells as factories for their own replication and have the ability to display peptides or proteins on their surfaces. This technology is called phage display. Phage display can be used as a powerful tool to screen for affinity reagents for all kind of targets, ranging from small molecules to proteins and even cells. This selection can be performed by using phage libraries consisting of a high number of different phages ($10^8$ - $10^{10}$), each displaying a different peptide or protein on its surface. Among the huge number of phages in these phage libraries, the ones with high affinity and specificity for a target can be isolated in an affinity selection procedure (Fig. 1). Moreover, the proteins and peptides displayed on these selected phages can be identified by sequencing the gene coding for the displayed protein or peptide. This coding gene can be found in the single-stranded DNA (ssDNA) inside the selected phage. The target specific phages can be used as affinity reagents in diagnostic tests. Besides the target specific phages, the soluble peptides or proteins, released from the phage coat, can also be used as affinity reagents. These peptides or proteins are then produced synthetically or by recombinant expression in bacterial cells.

Different types of phage libraries exist, displaying different types of peptides or proteins: peptides, cellular proteins (from cDNA libraries) or antibody fragments, like single chain variable fragments (scFv) and antigen binding fragment (Fab). **Antibody fragment phage libraries** are used commonly in immunology (Hoogenboom et al. 1998). Their wide diversity enables them to imitate the natural immune system. Phage display enables the production of sizeable amounts of the affinity reagents, avoiding the batch to batch

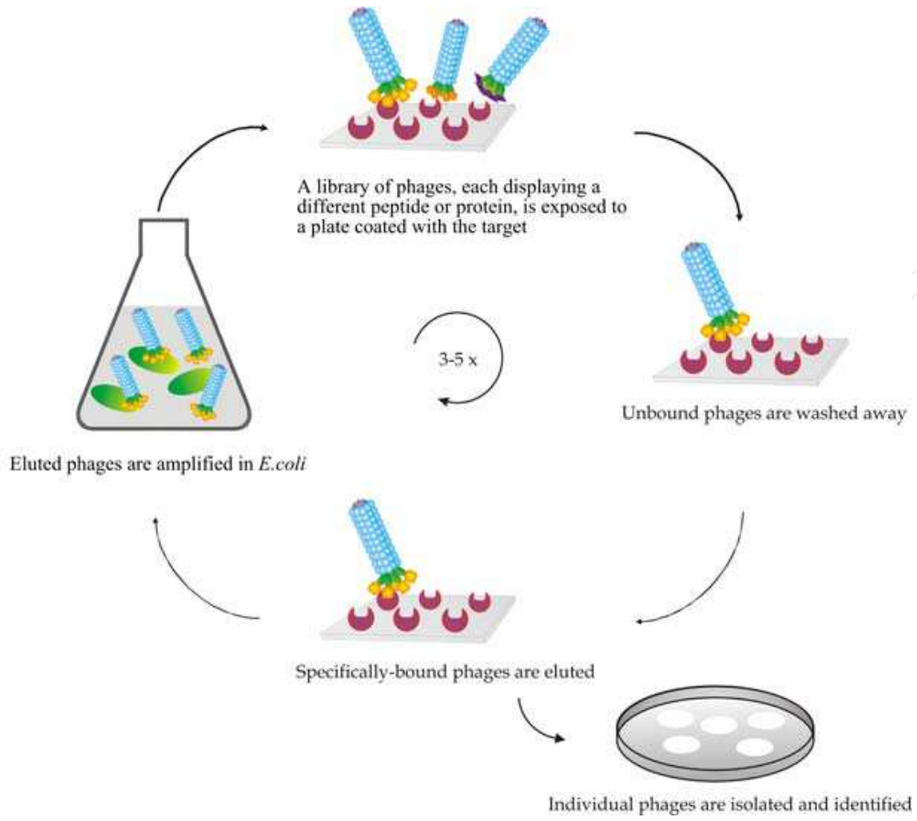


Fig. 1. Schematic representation of the phage affinity selection procedure (Van Dorst et al. 2010a)

variations which occur with classical antibodies. Moreover, there is no immune response required to produce affinity reagents for a target, making the selection of affinity reagents for poorly immunogenic targets possible. **Peptide phage libraries** can also be used to select affinity reagents, since the binding site, or epitope, only involves a few amino acids. Besides the monovalent phages that display one peptide on the phage surface, so-called landscape phages are also used as affinity reagents. These landscape phages display thousands of copies of peptides in a dense, repeating pattern around the tubular capsid (Petrenko & Smith 2000). The display of these thousands of peptides gives the phage surface different characteristics. The binding affinity of the landscape phages for the target is not determined by the affinity of one of these peptides alone, but by the whole structure. In **cDNA phage libraries**, cellular proteins are displayed on the surface of the phages. These cDNA phage libraries are frequently used for protein interaction studies (Crameri & Kodzius 2001; Pelletier & Sidhu 2001; Li & Caberoy 2010; Van Dorst et al. 2010c). Furthermore, they can be used as affinity reagents in diagnostic tests to detect antibodies for cellular proteins and to diagnose autoimmune diseases.

## 4. Aptamers

Aptamers are small sequences of nucleic acids, RNA or DNA, and more rarely peptide chains, able to specifically recognise a given target, ranging from small molecules to entire organisms (bacteria). The term *aptamer* derives from the Latin word *aptus* meaning *fitted* or

*suitable,* and the Greek word *meros* meaning *part* or *portion,* referring to the folding properties of single-stranded nucleic acids, responsible for their specific tridimensional structure. This hairpin structure can bind targets so tightly that some aptamers can even differentiate between two isomers, or closely related molecules. For instance, an aptamer selected for theophylline has shown that its binding affinity was 10,000-fold more important for theophylline than caffeine, only differing in structure by a methyl-group (Jenison et al. 1994). In this application of nucleic acids in biotechnology, the recognition is based on the tridimensional structure of the aptamer, more than its sequence. Aptamers undergo significant conformational changes upon target binding, thus offering great flexibility in design of novel biosensors; for instance, being formatted into molecular beacon structures (Yamamoto et al. 2000a).

Aptamers are selected from synthetic nucleic acid libraries which can contain more than $10^{15}$ different sequences, by an *in vitro* selection procedure commonly called SELEX, an acronym for Systematic Evolution of Ligands by EXponential enrichment (Stoltenburg et al. 2007). This iterative process consists of binding and elution steps of aptamers in contact with the molecule or organism of interest, copying the resulting aptamers by using PCR and finally separating the two strands obtained after the polymerisation (Fig. 2). After each cycle, the pool is enriched and used for the next selection cycle, until the amount of eluted aptamers reaches 80 to 90% of the initial amount of the pool. Usually, eight to fifteen selection cycles are needed to isolate aptamers with a high binding potential. Once a specific aptamer is selected for a target, this recognition element can be integrated into a transducing structure or device, in order to construct a biosensor.

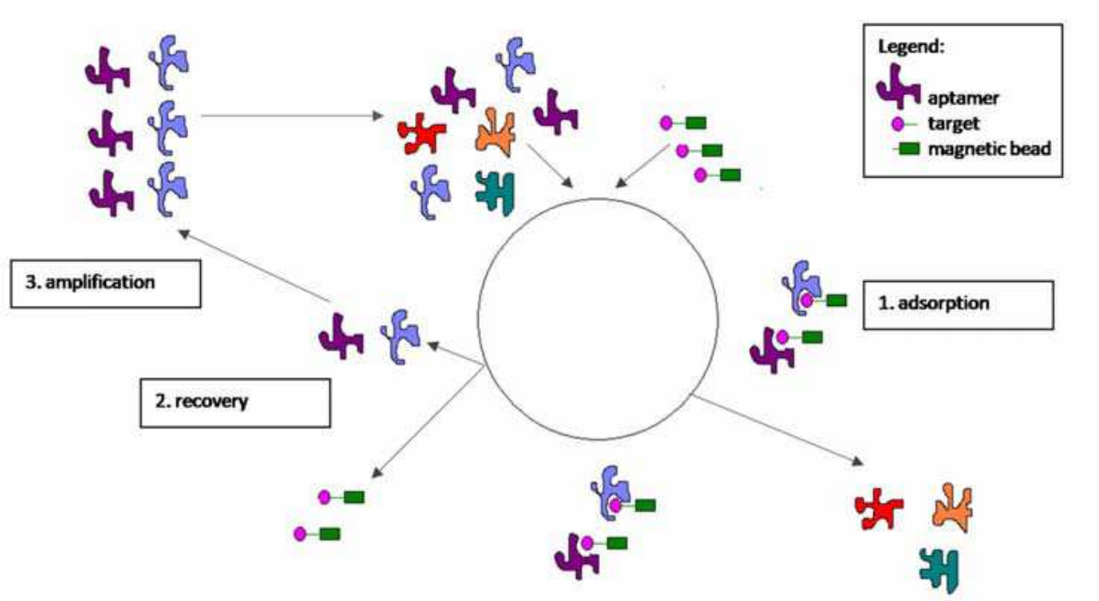


Fig. 2. Selection of aptamers via SELEX procedure (Van Dorst et al. 2010b)

## 5. Advantages and limitations of phages and aptamers

The use of both, phages and aptamers, as affinity reagents in diagnostic tests have some distinct advantages:

- High sensitivity and specificity for targets

Phages and aptamers are selected from their respective libraries which have a very wide diversity, even higher than that of antibody repertoires screened with the hybridoma technique. Strong selection conditions and counter selections with related targets result in specific binding phages and aptamers with high affinity. Moreover, the sensitivity and specificity of the selected phages can be increased with genetic modification (Sheedy et al. 2007). On the other hand, the selection process for aptamers can be directed to recognise specific structural or chemical motifs of the target, which is not always possible with antibodies. Phages and aptamers can both recognise their targets in ranges below nanomolar amounts, sometimes even down to picomolar ranges (Collett et al. 2005; Zhu et al. 2008).

- Fast, inexpensive and animal-friendly production

Phages are produced by infecting bacteria, a process which takes only a few hours and no animals need to be immunised; nucleic acid production is completely animal-free too, as it is chemically synthesised on a large scale. Thus both techniques avoid batch-to-batch variations, are very cost-effective and are reproducible.

- Stability

Phages are stable in a wide variety of conditions: at high temperatures (Brigati & Petrenko 2005) and in a wide pH range from 3 to 11 (Jepson & March 2004; Verma et al. 2009). Moreover, phages can even resist nucleases or proteases (Schwind et al. 1992). Their high degree of stability not only offers technical, but also economic advantages. The degree of stability extends the lifetime of diagnostic tests and reduces transport costs by avoiding the need for cooled shipment and storage.

Nucleic acids allow the use of a wide variety of buffers and organic solvents, which can be interesting for monitoring organic compounds. Indeed, nucleic acids remain stable in hydrogen bond forming solvents such as ethylene glycol, methanol, formamide, dimethyl sulfoxide or acetamide (Bonner & Klibanov 2000). As opposed to their protein counterparts, aptamers can be selected under nonphysiological conditions or real matrix conditions, which is particularly useful for biosensing clinical and food samples.

- Ease of modification

Phages are easily modified, because of the accessible amine groups on the phage coat protein VIII. Via these amine groups, phages can be immobilised or conjugated to labelling molecules (Jin et al. 2009).

Aptamers can easily bear labels at each end of the strand. For instance, reporter molecules such as enzymes or fluorophores, were used in the development of aptamer biosensors (Tombelli et al. 2005b). A wide variety of labels can also be used with nucleic acid to be immobilised onto different surfaces. Nucleic acid biotinylation is a technique widely spread in biotechnologies, based on the strong bond between biotin and avidin (or streptavidin). This allows nucleic acids to be fixed to almost any type of streptavidin-modified surfaces, which are in many cases commercially available. Alternatively, nucleic acid can be labelled with phosphate, amine or thiol moieties enabling a covalent immobilisation of the nucleic acid probe.

- Reusability

This is an advantage unique to aptamers. Unlike antibodies, aptamers can undergo several cycles of denaturation and regeneration. This allows aptasensor platforms to be recyclable and reusable.

Though phage and aptamer technology hold great promise, there are limitations:

- Unavailability of standardised protocols: For each new target, specific modifications are usually necessary. Moreover, protocols have to be adjusted and parameters often have to be optimised before the selection technique offers significant results, for both phages and aptamers.
- Immobilisation of targets: A requirement for phage and aptamer selections is that the targets need to be immobilised on a solid support; this is easily achieved for proteins and whole cells but is more complex for smaller molecules where specific functionalisations are necessary to enable efficient immobilisations.
- Nuclease sensitivity: Nucleic acids can be degraded in real matrices by the action of nucleases. This concern can be overcome by chemical modification of the ribose ring at the 2′ position with fluorine or amino groups. Another technique consists in using mirror-image analogues, called spiegelmers, which are nuclease resistant (Eulberg & Klussmann 2003)

## 6. Applications of phages

### 6.1 Medical diagnostics

#### 6.1.1 Autoantibody detection in diseases characterised by deviations of the immune system

cDNA and peptide phage display are applicable for epitope mapping of monoclonal or polyclonal antibodies. In medical practice, this epitope mapping approach has potential value in the profiling of circulating antibodies in diseases characterised by immune system deviations, such as cancer and autoimmune disorders; the analysis of circulating antibodies allows the identification of immune-targeted tumour antigens in cancer and auto-antigens in autoimmunity. Depending on the affinity selection approach employed, disease-specific circulating antibody reactions can be used as diagnostic markers, as markers for prognosis and therapeutic outcome, and as markers for therapeutic responsiveness. The selected peptide or protein displaying phages can be used in diagnostic tests to detect disease-associated or disease-specific antibodies. Moreover, the identified antibodies and associated antibody-targets can be important for the discovery of novel therapeutic targets in cancer and autoimmunity research.

Serological antigen selection (SAS) is a phage display-based autoantibody profiling approach in which a cDNA expression library from diseased tissue or cell-lines is displayed at the surface of phage particles, followed by affinity-selection with pooled patient antibodies (Fig. 3). This procedure has recently been successfully applied for colorectal cancer (CRC) (Somers et al. 2002), atherosclerotic lesion rupture (Cleutjens et al. 2008), and multiple sclerosis (MS) (Somers et al. 2008; Govarts et al. 2009).

The SAS procedure entails subsequent rounds of affinity selection of a cDNA phage display library with patient immunoglobulins. An affinity selection round is initiated by incubating phage displaying the cDNA library with pooled patient body fluids (1). During this incubation step, antigen-antibody complexes are formed between the antibodies present in the patient body fluids and their respective target antigens at the surface of the phage (2).

These complexes are captured on a solid support by anti-human IgG capture antibody (3) while non-bound phages are washed away (4). Bound phages are eluted (5), amplified through infection of host bacteria (6) and used as input in a subsequent affinity selection procedure (7). The succession of affinity selection and amplification of selected phages results in enrichment op phage displayed antigens targeted by patient IgG.

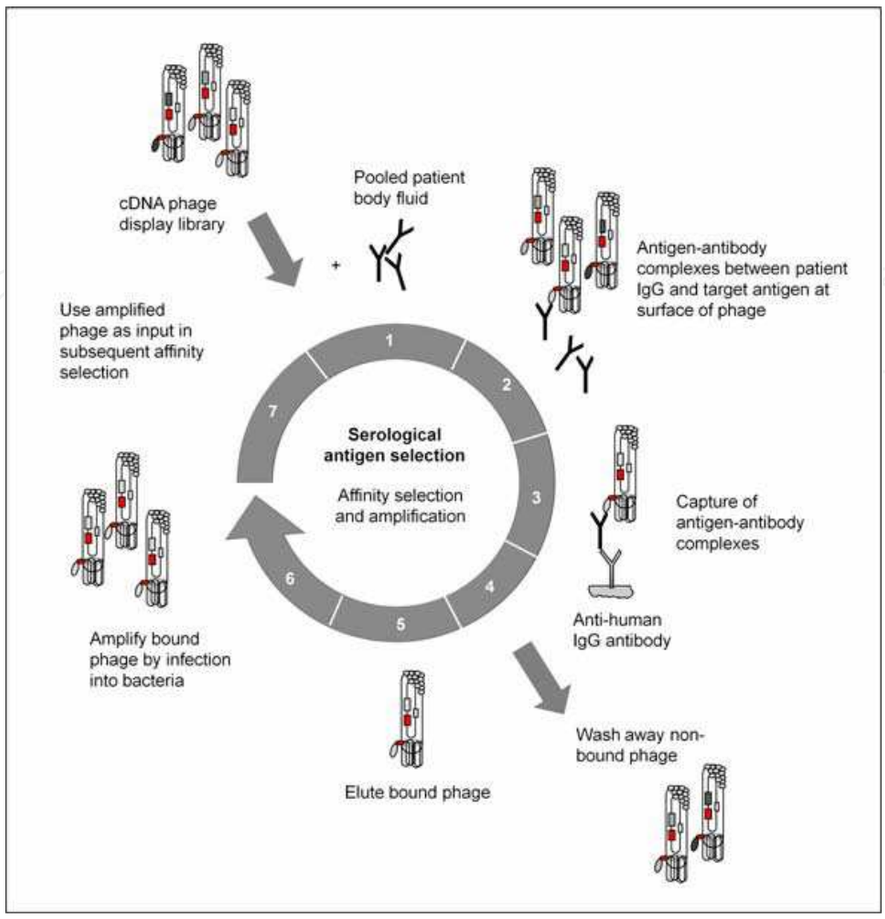


Fig. 3. Overview of the serological antigen selection (SAS) procedure

The SAS technology was initially developed to discover novel candidate tumour-antigens for CRC. In this study, the procedure was applied on a cDNA expression library from CRC cell line HT-29 using pooled CRC patient serum. Six phage clones with a CRC-related serological profile were identified, making excellent candidates for tumour vaccination and sero-diagnosis of cancer (Somers et al. 2002).

For the identification of novel markers for ruptured atherosclerotic lesions, SAS was performed on a cDNA expression library prepared from mRNA preferentially expressed in ruptured human atherosclerotic plaques. Sera from patients with ruptured atherosclerotic plaques were used for selection of phages expressing immunoreactive fusion proteins resulting in the identification of two phage clones. The presence of antibodies against the peptides displayed by these clones could discriminate between patients with peripheral ruptured lesions and patients with peripheral stable plaques with 100% specificity and 76% sensitivity. A positive serum response against a set of two novel identified cDNA phage clones thus showed a strong association with the presence of ruptured peripheral atherosclerotic lesions, allowing a promising novel approach for non-invasive diagnosis of atherosclerotic lesions (Cleutjens et al. 2008).

In another study, the use of the SAS procedure on a cDNA expression library from MS brain tissue and selection on pooled cerebrospinal fluid (CSF) of MS patients has led to the discovery of eight novel phage clones and associated autoantibody reactions with 45%

sensitivity and 86% specificity for the disease. As currently the diagnosis of MS is largely based on clinical symptoms, the implementation of objective laboratory tests that can measure the serological presence of the markers can be of great value to MS diagnostics. Moreover, the analysis of the MS-associated immune deviations through autoantibody profiling is also valid to provide the urgently needed clues regarding disease-associated autoantibody-targets and the underlying autoimmune aetiology (Somers et al. 2008; Govarts et al. 2009).

Phage-based biosensor platforms that incorporate the novel identified cDNA phage clones can be developed for serological screening of patients, resulting in clinical application of these phage clones in medical diagnostics.

### 6.1.2 Serum diagnosis of infectious disease by detection of antibodies with a phage-particle gel immunoassay (PPaGIA)

Infectious diseases are a matter of national security and may have a great impact on public health and the world economy, especially due to rapid dissemination and adaptation of pathogens. Proper diagnosis of infectious diseases with reliable, robust and sensitive tests is very important for individual health, but is also crucial for surveillance and control of infectious diseases worldwide. One possible diagnosis of infectious diseases is the detection of pathogen-specific antibodies in the blood or serum of patients. Phages are very appropriate for the development of these antibody detection tests. Peptide phages displaying a peptide that mimics the antigen (mimitope) and binds to antigen-specific antibodies, can be selected. Peptide phage display is already widely used for epitope mapping of poly- and monoclonal antibodies (Irving et al. 2001). However, the selected peptide phages that bind to pathogen specific antibodies in these studies can also be used in diagnostic tests to detect these pathogen-specific antibodies. Goulart et al. (2008) developed a phage-particle gel immunoassay (PPaGIA), which uses polystyrene beads conjugated with peptide phages with affinity for pathogen-specific antibodies. When pathogen-specific antibodies are present in blood or serum of infected patients they will bind to the beads conjugated with these peptide phages. These antibody-bead complexes can be separated by size from the beads that do not interact with the antibodies, by centrifuging through a gel matrix of chromatographic microcolumns. When antibody-bead complexes are formed, they are captured at the top of the column, in contrast with plain beads that will be detected at the bottom of the column after centrifuging. An example of leismaniasis diagnosis with PPaGIA is visualised in Fig. 4.

### 6.1.3 Metastatic cell detection

Metastasis is the spread of cancer from the place at which it starts as a primary tumour to other tissues in the body. An early detection of metastasis is important for early treatment of cancer, which increases the chances of a cure. The current diagnosis of metastasis is only possible by a series of biopsies in different tissues. However, biopsy is an invasive approach, which is not applicable in the early stage of metastasis and less applicable if patients do not have a clearly defined pathological site (Aggarwal et al. 2005). A promising alternative for metastasis diagnosis is the measurement of tumour cells in the blood, because the metastatic tumour cells are loosened and released into the blood or lymph. For the development of diagnostic approaches to detect metastatic tumour cells in blood, affinity reagents selective for metastatic markers, unique to tumours are required. Here, phage display can be used. A number of peptides that bind specific metastatic tumour cells are identified by differential

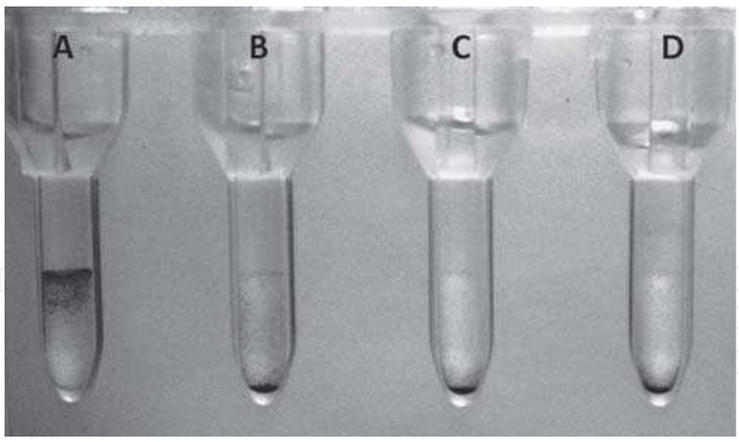


Fig. 4. Digital image of the PPaGIA. Circulating antibodies from leismaniasis patients were detected with peptide phages that specifically recognise antibodies for visceral leismaniasis (VL). A: positive serum from a patient with VL detected with specific phages (agglutination at the top); B: positive serum from a patient with cutaneous leismaniasis (CL) probed with specific phages (no reaction); C: positive serum from a patient with VL probed with nonspecific phages (no reaction); D: negative serum probed with specific phages (no reaction). (Goulart et al. 2010)

screening with phage display (Rasmussen et al. 2002; Jia et al. 2007; Rittner et al. 2007; Zhang et al. 2009). The selected metastatic specific peptide phages can be used to develop a diagnostic approach to detect metastatic cells in blood samples. Zhang et al. (2009) developed a light-addressable potentiometric sensor (LAPS), that measures the surface potential of an illuminated sensor chip. The metastatic-specific peptide phages are covalently coated on the sensor chip of this LAPS, as visualised in Fig. 5.

The blood sample is injected in the microchamber and metastatic cells are captured by the immobilised phages. This capturing of metastatic cells causes changes in surface potential, which is measured and translated in an output voltage with the lock-in amplifier. With this LAPS modified with metastatic-specific peptide phages, it was possible to distinguish between metastatic cells and non-metastatic cells and detect as few as 100 metastatic cells per ml. This approach shows the potential of peptide phage display to develop diagnostic approaches for metastatic cancer.

### 6.2 Food biosensors

#### 6.2.1 Salmonella typhimurium detection

*S. typhimurium* is one of the leading sources of human gastroenteritis, causing mainly diarrhoea, vomiting and abdominal cramps 12 to 72 hours after infection and possibly lasting for up to seven days. Humans are usually infected with *Salmonella* by ingesting contaminated food such as raw meat, fish, unpasteurised dairy products and eggs. A conventional, accurate way to detect *S. typhimurium* in food samples can be carried out by means of microbiological culture growth techniques. The problem, however, is that the results are only obtained within three to five days. For this reason, several rapid DNA-based (McGuinness et al. 2009), biochemical, immuno-assay (Beumer et al. 1991) and immuno-latex agglutination methods have been developed. Mostly, they obtain results within 48 hours. Still, faster detection of *S. typhimurium* is necessary to avoid outbreaks by preventing

infected food from being distributed and consumed. Biosensor technology can provide such rapid results.

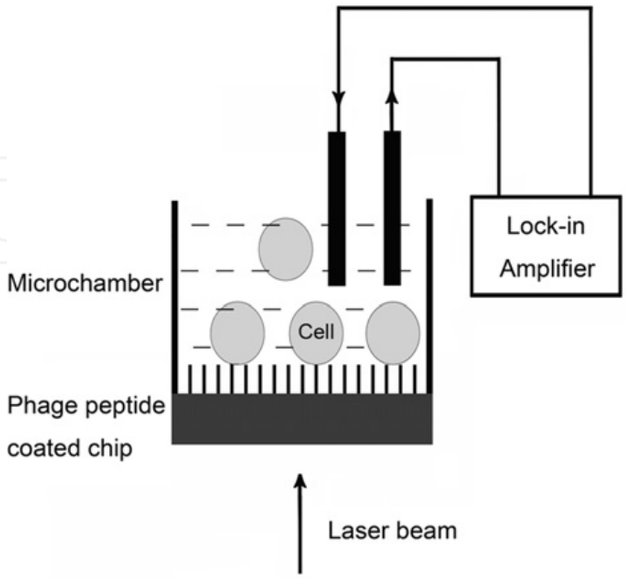


Fig. 5. Schematic representation of LAPS modified with metastasis specific peptide phages (Zhang et al. 2009)

A Magnetostrictive Microcantilever (MSMC) was developed as a transducer for the development of a biosensor for *S. typhimurium* by Fu et al (2007). The technology is based on applying a time-varying magnetic field, on the cantilever (Fig. 6). A filamentous peptide-displaying phage against *S. typhimurium* was used as a biorecognition element, which was immobilised on the surface of the cantilever by direct physical absorption. The resonance frequency, registered by two pick-up coils connected to a lock-in amplifier to measure the electric potential, shifts with time as the target bacterium binds on the sensor surface. The resonance characteristics of the MSMC in liquid were studied and the device proved to function well in water.

A similar sensor, based on the application of a magnetic field was built by (Lakshmanan et al. 2007) for the detection of *S. typhimurium*. The phage was derived from a landscape phage library. Instead of using a cantilever, a piece of ribbon on which the phages were immobilised, was utilised (Fig. 7) and the analyte was passed over the sample using a peristaltic pump. A detection limit of 103 cfu/ml was obtained for the sensor. The main advantages of these sensors are the sensitive and real-time detection.

The same approach of detection with magnetoelastic biosensors was used by different authors (Wan et al. 2007; Huang et al. 2008; Johnson et al. 2008) for *Bacillus anthracis*. Olsen et al (2006) developed a Quartz Crystal Microbalance (QCM) biosensor for the detection of *S. typhimurium*. QCM sensors are based on the resonation of the quartz crystals on application of an external alternation electric field. The frequency of the resulting oscillation is a function of the mass of the crystal. When the target binds to the phages, which are immobilised on the metallic electrodes on both sides of the piezoelectric quartz crystal, a shift in resonance frequency of the quartz crystal occurs. The sensor delivered a rapid response (< 180 s) and had a low-detection limit of 102 cells/ml.

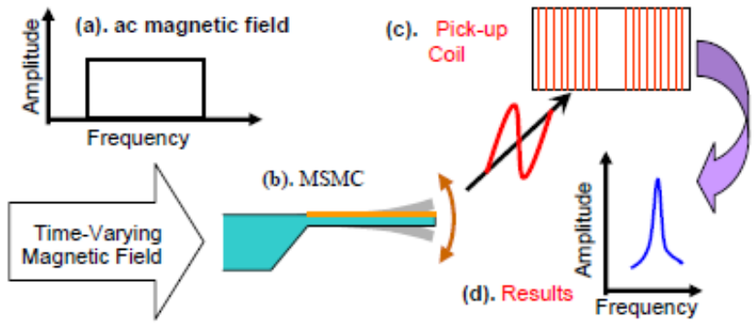


Fig. 6. Schematic illustration of the principle of MSMC as a transducer for biosensors. (Fu et al, 2007)

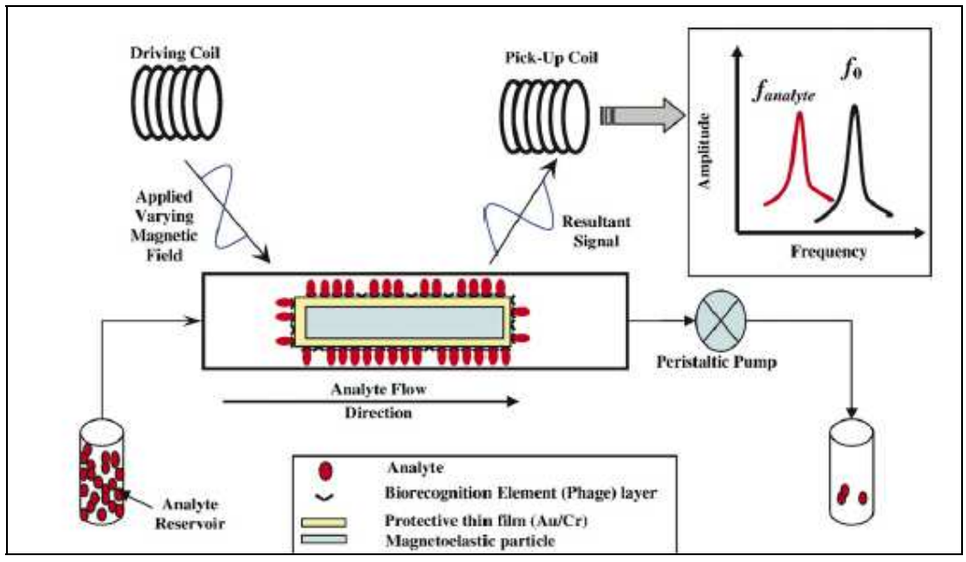


Fig. 7. Scheme illustrating the wireless nature of the magnetoelastic biosensors and the basic principle for detecting bacterial cells. The binding of bacterial cells is on both sides of the sensor (Lakshmanan et al. 2007)

### 6.2.2 Detection of Staphylococcal enterotoxin B (SEB)

SEB is produced naturally by the *Staphylococcus aureus* bacterium in improperly refrigerated, stored, and handled foodstuffs and is a very common cause of food poisoning. The toxin can be ingested or inhaled and can be used as a warfare agent. The clinical signs include symptoms such as headache, fever and chills. Depending on the route of exposure, additional symptoms can be observed. Ingestion results in nausea, vomiting and diarrhoea while inhalation provokes non-productive cough, chest pain and dyspnea. Classical ways of detection are mainly based on multiplex PCR (McLauchlin et al. 2000; Mehrotra et al. 2000) and immunoassays (Freed et al. 1982; Wieneke 1991), which allow quantitative or qualitative measurements in less than 24 hours.

A peptide displaying-phage for the optical detection of SEB with biosensor technology was developed by Goldman et al (2000). The SEB binding phage was selected from a random

peptide library and was fluorescently labelled with Cy5 dye to allow detection. The labelled phages were among other methods tested in a portable fibre optic biosensor (RAPTOR) (Fig. 8). Four optical probes, placed in a disposable chamber were coated with SEB or streptavidin, and the labelled phage was loaded onto the chamber. The complex formation could be monitored by the surface-bound fluorophores with a diode laser. The optical probe captures a portion of the emitted fluorescence, which travels back up the fibre to the photodiode detector (Anderson et al. 2000). Different laser readings were taken at different moments of the one-hour assay. The phage did generate a robust signal however it gave a lower signal than that generated by a labelled antibody and the detection of small amounts of SEB was problematic. Nevertheless, in optimised forms, this approach has the potential to perform at levels comparable to their antibody counterparts.

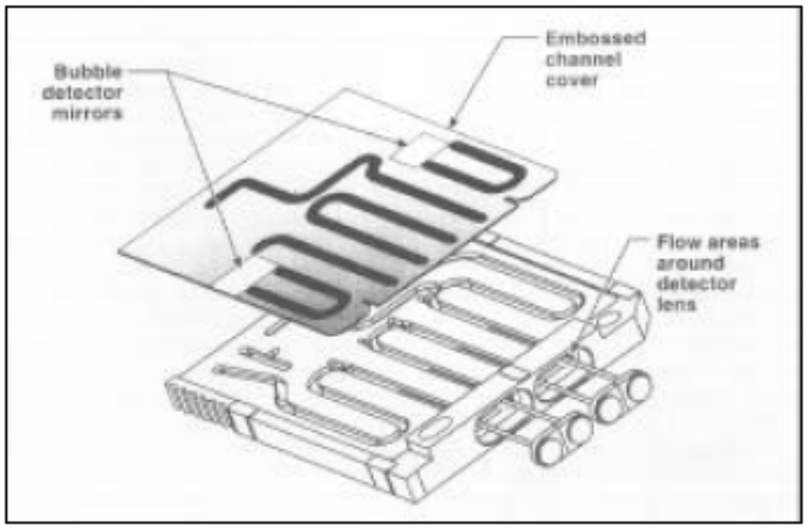


Fig. 8. Schematic of the RAPTOR showing four fibre optic probes, adhesive channel cover and bubble detector mirrors (Anderson et al. 2000)

## 7. Applications of aptamers

### 7.1 Medical diagnostics

#### 7.1.1 Electrochemical detection of Platelet-derived growth factor (PDGF)

PDGF is a protein that regulates cell growth and division (Degefa & Kwak 2008) and is believed to be differentially expressed in human cancer (Polanski & Anderson 2006). Tumour cell lines produce and secrete PDGF (Famulok & Mayer 1999), thus making it a plasma biomarker that could be useful in early cancer detection and monitoring. This growth factor also participates in other proliferative disorders such as glomerulonephritis (Iida et al. 1991) and arteriosclerosis (Lindner et al. 1995; Lindner and Reidy 1995). Green et al (1996) identified DNA aptamers binding to PDGF (AB and BB variants) with subnanomolar affinity and which inhibit the mitogenic effects of PDGF. Using the highest binding aptamer, Lai et al. (2006) fabricated and characterised an electrochemical, aptamer-based (E-AB) sensor for the detection of PDGF directly in blood serum. The E-AB approach uses alternating current voltammetry to monitor target induced folding in a methylene blue (MB)-modified, PDGF binding aptamer. The E-AB sensor is constructed by attaching a MB-

modified, PDGF-binding aptamer to a gold electrode via self-assembled monolayer chemistry. In the absence of its target (Fig. 9, left), the aptamer is thought to be highly dynamic and partially unfolded, retaining only one of the three stems intact. This reduces electron transfer between the electrode and the MB. Upon target binding (Fig. 9, right), the aptamer folds into a configuration, forming a stable three-way junction, forcing the MB label into close proximity with the electrode, leading to improved electron-transfer efficiency.

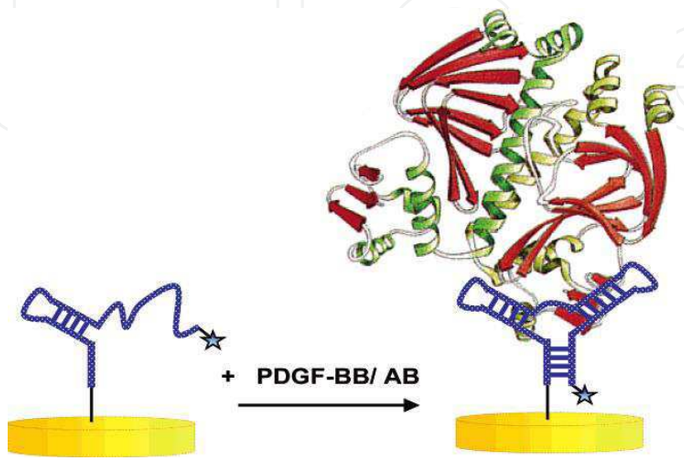


Fig. 9. E-AB-based PDGF sensor fabricated by a MB-labelled aptamer on a gold electrode surface (Lai et al. 2006)

This E-AB sensor combines the unprecedented sensitivity, selectivity and reusability of aptamers in a single approach, enabling it to detect physiologically relevant PDGF levels directly in largely unprocessed clinical samples, for the first time. It readily detects the BB variant of PDGF till 1 nM directly in undiluted blood serum and at 50 pM in serum-diluted two-fold with aqueous buffer. The detection limit attained in 50% serum is achieved against a >25 million-fold excess of contaminating blood proteins and represents a four order of magnitude improvement over the most sensitive optical PDGF aptasensor reported to date (Fang et al. 2001). This detection limit enables the sensor to easily measure 400-700 pM serum PDGF concentrations, from low levels found in healthy individuals to higher concentrations in cancer patients (Leitzel et al. 1991). The E-AB sensor's selectivity was studied using two control sequences sharing similar sequence identity and similar structure to the PDGF binding aptamer, respectively. However, both these sequences exhibited an insignificant signal increase (< 1%) under the same experimental conditions. Furthermore, the E-AB sensor is reusable and could be used and regenerated up to five times before unacceptable degradation was observed. Additionally, as an electrochemical sensor, the E-AB platform does not require light sources, high-voltage power supplies, or other heavy, cumbersome equipment. Given these advantages, E-AB sensors appear well suited for implementation in portable convenient point-of-care diagnostics, enabling direct detection of proteins and small molecules in complex, unprocessed clinical samples.

### 7.1.2 HIV -1 Tat protein detection using molecular beacon aptamer

Transcription of human immunodeficiency virus type-1 (HIV-1) is enabled via cellular factors and viral proteins (Gaynor 1992). Trans-Activator of Transcription (Tat) is an HIV-1 RNA-binding protein that exhibits an inherent affinity against the Trans-Activating

Response Element (TAR) of the virus and regulates viral transcription (Tombelli et al. 2005a). For HIV-1, oligonucleotides have been isolated that can efficiently interrupt essential steps in the viral life cycle, opening up the potential of treating viral diseases with aptamers (Famulok & Mayer 1999). A novel RNA motif that binds efficiently and specifically to the HIV-1 Tat protein and inhibits the trans-activation by Tat of transcription *in vitro* and *in vivo*, has been selected (Yamamoto et al. 2000b). This aptamer featured a similar structure to TAR and exhibited a 133-fold increased affinity for Tat, compared to TAR. Moreover, the aptamer does not require a cellular protein for efficient binding to the Tat, unlike the TAR of HIV-1. Hence the aptamer is not only useful for inhibiting the Tat function *in vivo* but also serves as a diagnostic reagent for the detection of Tat.

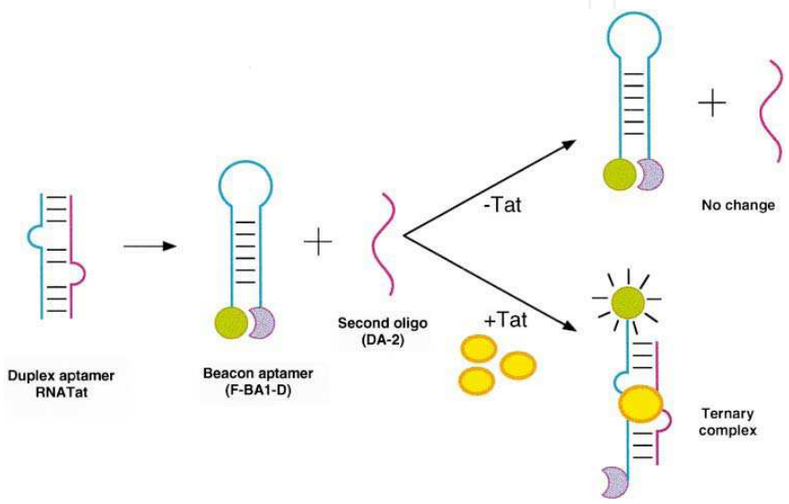


Fig. 10. Schematic representation of molecular beacon aptamer strategy for analysing the viral protein (Tat). (Yamamoto et al. 2000a)

To make a diagnostic test using a new molecular beacon aptamer, Yamamoto et al. (2000a) split the duplex aptamer into two RNA oligomers (Fig. 10). One of the split RNA oligomers (F-BA1-D), was designed to form a hairpin structure containing a fluorophore (fluorescein) and quencher (DABSYL) one at each end of the RNA strand and the other, DA-2, was a non-structured oligomer. It was observed that in the presence of Tat or its peptides (100 nM), the two oligomers underwent a conformational change to form a duplex that lead to relieving of fluorophore from the quencher, and thus a significant enhancement of the fluorescence of fluorescein, followed by the stabilisation of the ternary complex (Fig. 10). These results suggest that splitting of the aptamer into two oligomers does not affect the affinity of the oligomers to the Tat, as long as they possess the core binding sequence.

The molecular beacon aptamer (F-BA1-D) described here does not depend on the target sequence (DA-2) alone, but also depends on the target molecule (Tat protein of HIV) other than the nucleic acid.

As a specificity check, other RNA binding proteins were tested, but none of them enhanced the fluorescein intensity of the beacon aptamer. Thus the beacon aptamer responds specifically to the Tat protein or its peptides and is indeed suitable for specific detection of Tat protein, derived from either HIV-1 or HIV-2. Such a molecular beacon aptamer could find applications as an analytical tool for monitoring viral protein levels, both *in vitro* and in infected cells (such as HIV infected cells).

### 7.1.3 Mass based detection of Human IgE

An application of aptamers in biosensors was reported by Yao et al. (2009) to quantify immunoglobulin E (IgE), an antibody produced by the immune system under allergic reactions. A person who has an allergy usually has an elevated level of IgE in blood, therefore the quantification of IgE can considerably ease the clinical diagnosis of allergy-mediated disorders (Gokulrangan et al. 2005). Although the detection of IgE was already possible thanks to methods such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) (Peuravuori & Korpela 1993; Ollert et al. 2005), this aptasensor offers a fast and cheap detection method requiring less serum sample and reagents. The use of this aptamer as a recognition element of the sensor enables detection of concentrations as low as 2.5 μg/L of IgE in buffer and serum

In this sensor, a 5'-biotin modified nucleic acid aptamer has been fixed to an avidin modified gold layer of a quartz crystal microbalance device (QCM), providing a strong immobilisation of the recognition element. The piezoelectric sensor generates a frequency shift during the formation of the complex made by IgE and the aptamer. A linear relationship between the frequency (Hz) and the concentration of IgE has been observed, following the regression equation:

$$y = 1.03x - 0.06 \quad (1)$$

with x, the frequency, and y, the concentration of IgE in the range of 2.5-200 μg/l, the equation having a correlation coefficient of 0.996.

After the analysis of a sample, this piezoelectric aptasensor could be regenerated with 30 mmol ethylenediaminetetraacetic acid (EDTA) and reused ten times with the same chip without loss of function.

Finally, this piezoelectric biosensor showed a high specificity when tested with interfering proteins such as IgG or bovine serum albumin (BSA), which generated an insignificant frequency shift lower than 5% of the signal obtained for IgE.

### 7.1.4 Detection of insulin

Another example of application of nucleic acid aptamers in biosensors can be represented by the sensing of insulin by an aptameric enzyme subunit (AES) (Yoshida et al. 2009). Insulin is a key molecule in the regulation of glucose in blood (Khan & Pessin 2002). The concentration of insulin is lowered in patients suffering from type-1 diabetes because of a reduced production of this hormone. Therefore, sensing the concentration of insulin in blood or serum can help the detection of type-1 diabetes.

The AES is constructed by connecting an enzyme-inhibitor aptamer to a target molecule-binding aptamer, where thrombin is the enzyme (Fig. 11). In the presence of insulin, a structural change of the aptamer occurs, preventing the hybridisation to the enzyme-inhibitor aptamer. This results in activating the enzymatic activity of thrombin to degrade fibrinogen, present in the chip. The presence of insulin generates clotting of fibrinogen and can be monitored by this sensor.

## 7.2 Food biosensors

### 7.2.1 Detecting Campylobacter jejuni using fluorescent assays

An infection by *C. jejuni* is the most commonly reported cause of acute bacterial-mediated food poisoning. The disease is generally mild, but complications such as the Guillain Barré

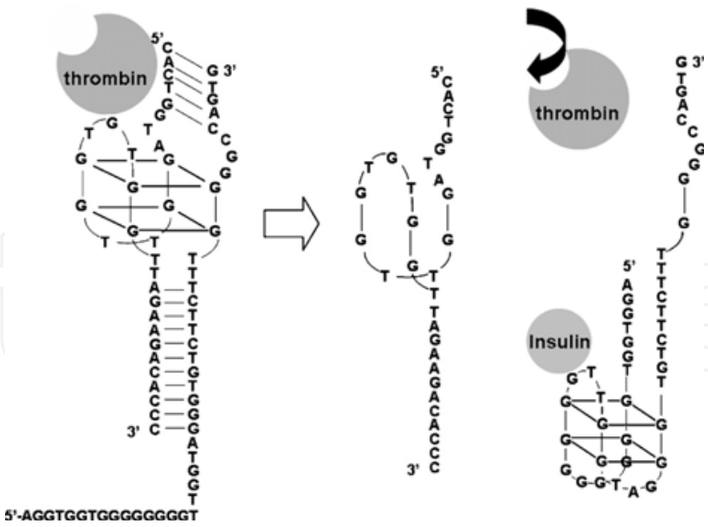


Fig. 11. Scheme of the AES-based insulin sensing. The target molecule-binding aptamer and a portion of its complementary strand were attached to the split thrombin-inhibiting aptamer. The nucleic acid aptamers are separated in the presence of insulin (Yoshida et al. 2009)

syndrome can occur. Remarkably, *C. jejuni* does not express a large number of so-called classical virulence factors. Additionaly, the role of the only verified *Campylobacter* toxin identified (cytolethal distending toxin) in *C. jejuni* has not been determined yet. The conventional culture detection method is time-consuming, while the more recent real-time PCR methods allow rapid and more reliable detection of the *Campylobacter* species. Bonjoch et al. (2010) used the gene encoding the ATP-binding protein CJE0832 to detect *C. jejuni* in a real-time PCR approach. Generally, even PCR-based methods need enrichment culturing to reach the desired detection limits.

Stratis-Cullum et al. (2009b) used the entire heat-killed *C. jejuni* cells as the selection target. A capillary electrophoretic assay was developed to investigate the relative binding affinity of *C. jejuni* aptamers. Fig. 12 illustrates the separation of the aptamer-bacterial cell complex, monitored by the emission of fluorescent labelled analytes at 520 nm. Migration depends on separation medium, size and shape of species, net charge on species and strength of electric field. The aptamer probe investigated, exhibited a pronounced mobility shift upon binding to the *C. jejuni* target, while a minimal response was obtained for two other food pathogen targets.

Bruno et al. (2009) developed DNA aptamers against the surface proteins of *C. jejuni*. The aptamers were used in a magnetic bead (MB) and red quantum dot (QD)-based sandwich assay, and the reactivity with bacterial species was evaluated with a spectrofluorimeter (Fig. 13). In this application the use of plastic cuvette-adherent technology improves detection of pathogens.

### 7.2.2 Electrochemical detection of Botulinum Neurotoxins (BoNTs)

Neurotoxins produced by *Clostridium botulinum* are among the most poisonous substances known. Food-borne botulism is the most common intoxication form due to the ingestion of pre-formed *Bo*NT in food. The gold standard method to confirm the presence of *Bo*NT consists of immunoassays, which are expensive, labour-intensive and slow. Recently,

alternative rapid methods, such as quantitative real-time PCR assays, have been developed for the detection of *C. botulinum* (Kirchner et al. 2010; Satterfield et al. 2010). Brunt et al. (2010) developed a sensitive and rapid immunochromatography column-based test for the detection of *Bo*NTs.

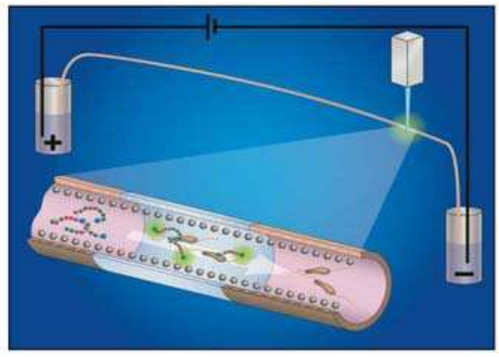

Fig. 12. Schematic representation of the capillary electrophoretic analysis of aptamer: bacterial cell complexes using laser-induced fluorescence detection

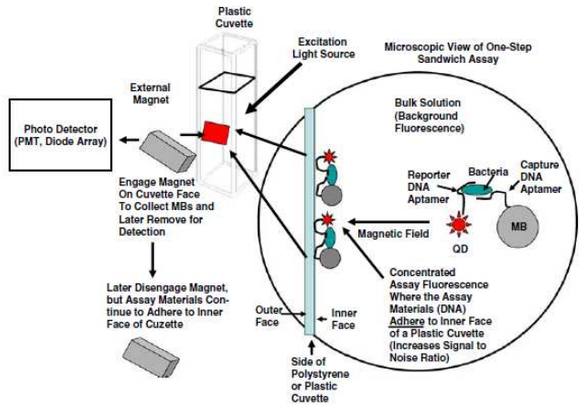


Fig. 13. Illustration of the self-assembling plastic-adherent aptamer-MB and aptamer-QD sandwich assay and magnetic collection system (Bruno et al. 2009)

Recently, Wei and Ho (2009) reported an electrochemical biosensor using an aptamer, which could measure *Bo*NT with a limit of detection of 40 pg/ml. The nucleic acid aptamers were selected via micro-bead SELEX procedure and their dissociation constants were determined to range between 3 nM and 50 nM, representing a quite good affinity towards *Bo*NT. When binding to *Bo*NT, a conformational change of the chosen aptamer was used for *Bo*NT monitoring (Fig. 14). A fluorescein label has been attached to one end of the aptamer in such a way that the label is hidden by the aptamer in the absence of the target. When *Bo*Nt is present in the sample, the aptamer unfolds and exhibits the fluorescein label, which can bind to an anti-fluorescein antibody linked to an enzyme, horseradish peroxidase (HRP), reducing hydrogen peroxide and oxidising tetramethylbenzidine (TMB); thus generating an electrochemical current signal due to the redox cycles between TMB, HRP and hydrogen peroxide. The specificity of the sensor has been proved by testing interfering proteins, which did not generate any electrochemical current, showing that the aptamer remained folded in the absence of *Bo*NT.

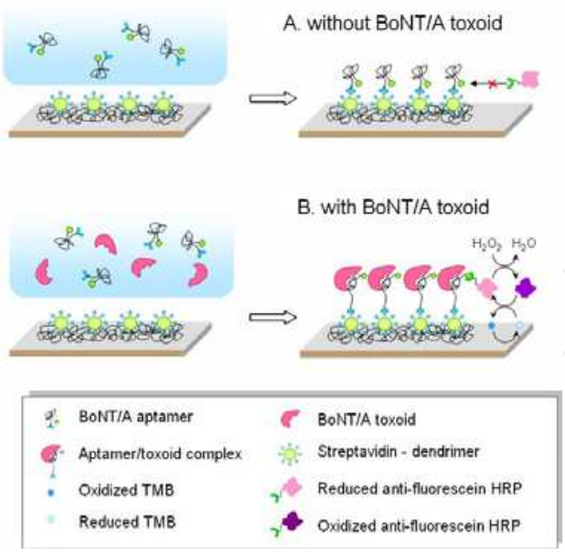


Fig. 14. Scheme of aptamer-based electrochemical detection of *Botulinum* neurotoxin (*Bo*NT) (Wei and Ho 2009)

## 8. Conclusions

Phages and aptamers have great potential as specific recognition elements in various biomedical and food applications. Their function as sensing receptors offers advantages over methods that are based on antibodies: their efficient immobilisation, the possibility of using different detection methods due to easy labelling and the easy regeneration of their functionality after immobilisation. Additionally, they can be modified, thus enhancing their stability, affinity and specificity and facilitating the functionalisation of nanoparticles and surfaces. These advantages enable the phage and aptamer biosensors to meet the requirements of sensitivity and stability that are necessary for the detection of disease biomarkers and food contaminants in their natural matrices.

The full potential of these novel biomolecules has not yet been realised completely and there are certain challenges that need to be overcome. However, proof-of-concept experiments demonstrating that aptamers and phages can specifically bind and regulate the function of various biomedically relevant targets, hold promise for the development of innovative diagnostics. The ability to develop affinity-based detection systems consisting of tailor-made characteristics, which can be applied to the analyses of disease biomarkers or carriers which are unlimited by size, complexity, toxicity and matrix effects, offers the field of biosensing the opportunity to explore new and dynamic routes of diagnostic sensor development. Overall, the potential of aptasensors and phage sensors is vast, and this exciting area of investigation is on the verge of exponential growth.

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