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Aptamer and its Potential Applications for Food Safety

Accompanied by industrial globalization, rapid urbanization, and population increment, mass production and staple trading for food consumption is upsoaring continuously, foodborne disease resulted from various food safety issues is currently an crucial public health concern worldwide, which has not only created a great burden on both economy and society, but also greatly threatened the sustainability of mankind livelihood and human reproduction. In order to better ensure food safety and thus effectively curb the occurrence of foodborne diseases, the development and evolving of inspection strategies are indispensable measures for quality assurance and conformity assessment. Nowadays, as complementary measures to and with advantageous merits over classic analytical methods, highly

specific and selective aptamer-based assays have found their increasingly important roles in various domains of food analysis. This critical review summarizes the advantages of aptamer as compared with antibody, introduces important evolving variants of systematic evolution of ligands by exponential enrichment (SELEX), and presents an overview of potential aptamer applications for food safety.

Keywords: Aptamer, SELEX, antibody, food safety, foodborne disease

1. INTRODUCTION

Accompanied by the industrial globalization, rapid urbanization, and population increment, mass production and staple trading for food consumption is upsoaring continuously nowadays, foodborne diseases becomes an increasingly important public health problem worldwide, and is a critical issue for health security and sustainability of both humanity and economy globally. Although developing and less developed countries suffer from the largest share of burden incurred by foodborne diseases, the percentage of the population suffering from foodborne diseases each year has been reported to be gradually increased up to 30% in industrialized countries. In USA, for instance, 1 out of 6 Americans or approximately 48 million cases of foodborne diseases, resulting in 128,000 hospitalizations and 3,000 deaths, are estimated to occur annually (CDC 2011 estimates).

Most of, if not all, the foodborne diseases are caused by various food safety issues originated from different physical, chemical or biological hazards as exemplified by microorganisms (e.g. Salmonella, listeria, *E. Coli* O157), heavy metals (e.g. lead, mercury), toxins (e.g. aflatoxin, ochratoxin A), pesticide residues, or veterinary drug residues, etc,.

Practically, illegal adulterations, the use of unauthorized additives, the utilization of lax standard or substandard food ingredients, and the production of faked foodstuffs, can resulted in food safety issues conclusively.

In order to eliminate foodborne diseases and safeguard consumer health, detection is the most crucial measure for food safety as in quality assurance and product conformity assessment process. Officially accredited classic analytical methods such as liquid or gas chromatography combined with mass chromatography, which are highly sensitive and selective normally, play important roles in the practice, but these methods are all laboratory controlled, time-consuming, expensive, and need well trained testing staffs. As complementary measures to aforementioned classical assays, antibody based analytical methods, such as enzyme-linked immuno-sorbent assay (ELISA) or gold immuno-chromatographic assay (GICA), which are rapid, sensitive, selective, and cost-effective for screening and on-site detection, are widely utilized to tackle various food safety issues, however, the robustness and adaptability of these antibody based methods are greatly compromised by the difficulty to raise antibody for toxicants or non-immunogens, the ethical problems of the use of animals, the time-consuming and costly production process, and non-specific polyclonal or unsteady monoclonal binding properties for in situ or real-time analysis, which necessitates the development and introduction of novel detection entity thereof.

This review will try to investigate the merits of aptamer for detection as compared with antibody, to introduce systematic evolution of ligand by exponential enrichment (SELEX) with its important evolving variants, and to present an overview of potential aptamer applications for food safety.

2. MERITS OF APTAMER OVER ANTIBODY

The use of antibodies for detection predates the 1950s, becomes widespread by the 1970s when polyclonal sera from immunized animals was popular, and prevails after the discovery of monoclonal antibody technology (Yallow, 1959; Kohler, 1975). Although various approaches, such as antibody humanization (Boulianne 1984; Vaughan, 1998), phage (Scott, 1990; Cwirla, 1990) or ribosome displaying (He, 1997; Hanes, 1998; Roberts, 1997), antibody engineering (Kirkham, 1999), and in vitro immunization (Van Ness, 1984), etc,. are developed to overcome limitations associated with antibody for detection as aforementioned, much more attention are being attached to the discovery and advancement of antibody alternatives nowadays.

The evolution of in vitro selection and amplification technique has allowed the discovery of specific nucleic acid sequences that bind to a wide range of heterogeneous target molecules with high affinity and specificity. The technique by which these oligonucleotide ligands are obtained is called systematic evolution of ligands by exponential enrichment (SELEX) process (Tuerk, 1990). The resulting oligonucleotide ligands derived from the Latin word “aptus” meaning “to fit”, are termed as “aptamers” (Ellington, 1990). As the name implies, aptamers are inherently suitable for detections instead of antibodies as molecular recognition elements.

Firstly, the selection and production of aptamer is an in vitro chemical process under a variety of flexible conditions with limited iterative rounds applicable for any targets, which ensures not only its prolonged shelf-life, but also batch to batch activity and physical stability, aptamer can easily recover its original conformation for temperature insulting and could be easily regenerated within minutes, are thus stable to long-term storage and can be transported at ambient temperature. To the contrary, antibodies are selected under physiological conditions,

have limited shelf-life, difficult to produce for toxins or non-immunogens, and raising monoclonal antibodies is normally time consuming and costly. Antibodies are temperature sensitive and undergo irreversible denaturation, its batch to batch quality and stability could not be easily guaranteed practically.

In addition, aptamers, as antibodies, can furnish an comparably superior binding ability with target molecules for sensitive detection, normally, the dissociation coefficients of aptamer-peptide or aptamer-protein conjugates are in low nanomolar to picomolar range, and in micromolar scale for aptamer-small molecule hyphenates, respectively. Both lengths and compositions of aptamer oligonucleotide sequences could be chemically adjusted on demand to get a better binding or detecting specificity, and the detection range is normally better than antibody based assays as well.

Furthermore, aptatope, which is a structural portion of a target molecule to which an aptamer binds, can be determined by an investigator for specific detection, and aptamers can be structurally modulated utilizing a toggle strategy to have a desired reactivity to detect multispecies when cross-reactivity is needed sometimes. Finally, aptamers are much easier to be chemically labeled or functionalized to procure better detecting adaptability.

The characteristic comparison of aptamers versus antibodies, on which the merits of aptamers over antibodies for detection are based, are summarized and tabulated in Table 1 herein.

(Table 1)

3. CONVENTIONAL SELEX PROTOCOL

SELEX is the acronym of systematic evolution of ligands by exponential enrichment as originally defined for the selection of RNA aptamers for T4 DNA polymerase (gp43) on nitrocellulose filters (Tuerk, 1990). As schematically shown in Fig.1, SELEX process to discover candidate aptamers for detection, which may ascribe to Darwin's theory of evolution, is characterized by in vitro selection cycles of binding, partition, and elution, repetitively.

(Figure 1)

A typical SELEX process is firstly to create a chemically synthesized random combinatorial oligonucleotide library consisting of about 10^{13} to 10^{15} sequence variants. Each variant in the library contains a sandwiched central random region flanked by different polymerase chain reaction (PCR) primer binding sequences. Typically, the central random regions of such libraries consist of 20 to 80 nt and primer binding sequences consist of 18 to 21 nt each. The selection of DNA aptamers and RNA aptamers almost shares the same protocol, but the DNA pool has to be converted into an RNA library prior to initiating the RNA SELEX process, that is, single-stranded DNA (ssDNA) library is converted into a double-stranded DNA (dsDNA) library by PCR, then dsDNA is transcribed in vitro by T7 RNA polymerase, resulting in a randomized RNA library.

In the first selection round, the library is incubated with the target molecule of comparably lower concentration, the resulting oligonucleotide-target complexes are partitioned from unbound and weakly bound oligonucleotides by rigorous washing steps, target-bound oligonucleotides are eluted and amplified by PCR (DNA SELEX) or reverse transcription PCR (RNA SELEX) subsequently. The resulting dsDNA is evolved into a new oligonucleotide pool

by purifying the relevant ssDNA or by in vitro transcription and purifying the synthesized RNA, and this enriched pool is utilized for target binding in the next SELEX round. Thus, by iterative cycles of selection, the initial random oligonucleotide pool is progressively reduced to obtain candidate aptamers with sequence information and the highest affinity, specificity for target molecule consequently.

The number of SELEX rounds depends on a variety of parameters, such as target property, library design, and partitioning method (e.g., affinity chromatography, magnetic separation, filtration, gel/capillary electrophoresis, flow cytometry, surface plasmon resonance, or centrifugation), etc. Normally, 6 to 20 SELEX rounds are required for aptamer selection. The core binding domains of aptamers typically range in length from 12 to 40 nt, for the ease of detection, most of the candidate aptamers can be further subjected to some post-modifications as to introduce functional groups, reporter groups, or spacers, etc.

Worthy of mention also is that referential negative selection or subtraction SELEX steps in most cases are needed to minimize the enrichment of non-specifically binding oligonucleotides or to direct the selection to an specific aptatope of the target molecule (Strehlitz, 2009).

4. IMPORTANT SELEX VARIANTS

SELEX with characteristic repetitive selection cycles is rapidly evolving nowadays to further meet detection needs or address various applicative issues, much efforts has been continuously made since the first introduction of SELEX in 1990 to rationalize oligonucleotide library, to improve aptamer selectivity, to expand target applicability, to enhance partition or

selection efficiency, etc. In recent years, many important SELEX variants are successfully developed as listed and described in Table 2, accordingly.

The readiness of the starting oligonucleotide library, which can furnish targets with sufficient structural diversity and specific binding or additional functionalities to enhance oligonucleotide stability of conformations or resistance to nucleases, is the foremost important factor for SELEX, both natural nucleic acids and chemically modified oligonucleotide libraries in either separated or combined format are used to rationalize starting oligonucleotide pools for aptamer discovery, as referred in the so-called genomic SELEX or cDNA-SELEX, Photo SELEX, phosphoro(di)thioate SELEX, Covalent- SELEX or Cross-linking SELEX, Tailored SELEX or Primer-free SELEX, Chimeric SELEX and Multistage SELEX, respectively.

In addition, as binding selectivity is the prerequisite for any aptamer-based applications, several SELEX strategies, such as negative SELEX, counter SELEX or subtractive SELEX, and deconvolution SELEX, etc., are utilized to improve candidate aptamer's binding selectivity by minimizing the co-selection of unwanted oligonucleotides, discriminating structurally related targets, selecting for a specific aptatope of a target molecule, or removing irrelevant oligonucleotides, consequently.

Furthermore, in order to improve aptamer adaptability or target applicability, conventional SELEX protocols are intentionally updated to screen aptamers against multiple targets or target entities, such as whole cell, whole bacteria, or even entire tissue, etc., which are termed as Complex target SELEX, Toggle SELEX, Blended SELEX, Expressions cassette SELEX or SELEX-SAGE, Mirror-image SELEX or Spiegelmer Technology, TECS-SELEX, Whole Cell-SELEX, Whole Bacteria – SELEX, correspondingly.

Also it's worthy to mention that SELEX variants such as Mono-SELEX, Non-SELEX or NECEEM-SELEX, CE-SELEX, Microfluidic SELEX, Automated SELEX or HTS-SELEX, FluMag-SELEX, etc., can obviously enhance SELEX partition efficiency by reducing selection cycles or improving screen throughputs accompanying with the advancing of contemporary analytical chemistry or separation science.

Recently, computational SELEX, which can be termed as in silico SELEX by the author, emerges as an important SELEX variant, this computational scheme can be used with either molecular docking scheme to reduce SELEX pool size (Yaroslav, 2009) or random filtering and genetic filtering algorithm to optimize structural complexities (Xuemei, 2011), in silico-SELEX with merits of both rational molecular simulation and high-throughput virtual screening will undoubtedly become an high efficient SELEX alternative for subsequent aptamer screening practice. New paragraph: use this style when you need to begin a new paragraph.

(Table 2)

5. APTAMER AND ITS POTENTIAL APPLICATIONS FOR FOOD SAFETY

5.1. Acute food pathogens

Acute food pathogens originated from the most contagious protein or virus can result in fatal damage to public health and regional economy, amongst which cellular prion protein (PrP) isoform of transmissible spongiform encephalopathies (TSEs) and H5N1 avian influenza virus (AIV) are the most notorious two, respectively.

A dual-aptamer strategy for highly sensitive differentiation of PrP isoforms and PrP detection is recently developed (Saijin, 2010), the scheme takes advantages of aptamers, magnetic microparticles (MMPs), and quantum dots (QDs). The aptamers, which were coupled

to the surfaces of MMPs and QDs, respectively, can recognize their corresponding distinct epitopes of prion proteins (PrP), based on which a sandwich structure of MMPs-Apt1-PrP-Apt2-QDs can be formed to discriminate PrP isoforms in serum and detect PrP in 0.01% brain homogenate successfully, the detection limit is about 1000-fold lower than that of commonly applied antibody-based assays.

(Figure 2)

In addition, for the detection of avian influenza virus (AIV) H5N1 gene sequence, a sensitive electrochemical method using a ssDNA aptamer immobilized onto a hybrid nanomaterial-modified electrode with superior selectivity and sensitivity is developed (Xianggang, 2011). The aptamer is synthesized with a thiol spacer for the ease of conjugation, the detection selectivity is based on the hybridization and conformational fitting of DNA aptamer with H5N1 specific sequence, a linear range of 5.0×10^{-12} to 1.0×10^{-9} M (R = 0.9863), and a detection limit of 4.3×10^{-13} M, are successfully acquired, respectively.

5.2. Dyes And Fungicides

Dyes or colorants are traditionally used in food industry for flavor perceiveness, nutrient conservation, visual uniformity, and other decorative or artistic purposes, some industrial dyes or food colorants, such as malachite Green (MG) and its primary metabolite leucomalachite Green (LMG), which are suspect human carcinogens, are routinely used as fungicides for aquaculture to protect fish and fish eggs against fungus infection.

A RNA aptamer with the sequence of 5'-GGAUCCCGAC UGGCGAGAGC CAGGUAACGA AUGGAUCC-3' is utilized to semi-quantitatively determine cationic triphenylmethane MG/LMG dye residues in fish tissues recently (Sara et al., 2010). The presence

of Mg^{2+} and K^{+} or Na^{+} is a requirement for the RNA complex formation and stabilization. Because the LMG metabolite is lipophilic and highly persistent in tissues, an oxidation step using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone as the oxidizing agent is incorporated within the sample cleanup procedure to ensure that all LMG residues are converted to MG prior to detection.

Another RNA aptamer against 4,4'-methylenedianiline, an azo dye which might exist in foods and a suspect human carcinogen , is developed using a typical SELEX protocol (Ulrike, 2004), the result has enabled the detection of DNA damaging compounds or DNA adducts eventually.

5.3. Antibiotics

Antibiotics, which are naturally produced or synthetically made antimicrobial agents, are widely used chemicals that in low concentrations can kill or inhibit the growth of bacteria without causing significant damage to host animal or human, non-human antimicrobial abuse in agro-food industry may result in high-level residual antibiotics in foods and incur severe human antimicrobial resistance thereof.

RNA aptamers against chloramphenicol are identified from two independent initial random RNA populations, a structural motif of 50 nucleotide RNA that is shared by selection sequences is identified as a minimal chloramphenicol aptamer consisting of a highly conserved central base pair helix flanked by A-rich bulges and additional helices (Burke, 1997).

DNA aptamer against tetracycline are identified and used for fast electrochemical determination of tetracyclines in milk (Zhang, 2010). The aptamer, which was immobilized on the surface of the glassy carbon electrodes, can specifically bind tetracyclines quickly in milk

and other samples for subsequent measurement. The linear relationship between the currents and the tetracyclines concentration is 0.1–100 ng ml−1, the sensitivity limit is 1 ng ml−1, and the detection time is only 5 min.

A highly specific ssDNA aptamer that binds to oxytetracycline (OTC) with high affinity is employed to discriminate other tetracyclines (Kim, 2009). the electrochemical signals generated from interactions between the aptamers and the target molecules is evaluated by cyclic voltammetry (CV) and squarewave voltammetry (SWV), respectively. The specificity for OTC is highly distinguishable from other structurally similar antibiotics. The dynamic range of OTC detection is determined to be 1–100 nM in semi-logarithmic coordinates.

Streptomycin-binding RNA aptamers with high affinity and specificity are isolated via in vitro selection and counter-selection using bluensomycin (Wallace, 1998). The binding site of streptomycin on the RNAs is determined via chemical probing with dimethylsulfate and kethoxal. The minimal size required for drug binding is a 46- and a 41-mer RNA for motifs 1 and 2, respectively. Using Pb^{2+} cleavage in the presence and absence of streptomycin, a conformational change spanning the entire mapped sequence length of motif 1 is observed only when both streptomycin and Mg^{2+} are present. Both RNAs require Mg^{2+} for binding streptomycin.

An RNA aptamer is proposed for faradaic-electrochemical impedance spectroscopy (F-EIS) detection of aminoglycoside tobramycin in human serum (González-Fernández, 2011), a partially and a fully O-methylated aptamer are evaluated and compared. The affinity constant for both aptamers is estimated by F-EIS and the selectivity towards other antibiotics is also studied. The analytical characteristics are evaluated using both aptamers and fully anti-tobramycin aptamer is selected for human serum experiments. Using a 1:0.5 dilution of the serum, a linear

range between 3 μM and 72.1 μM is obtained.

In addition, RNA aptamers against tobramycin, kanamycin and neomycin are identified with a much greater rate using a modified automated in vitro selection protocol (Goertz, 2004). The method is suitable for integration with high-throughput technologies, greatly expanding the possibility of discovering useful aptamers against other low weight targets.

5.4. Microorganisms

The existence of pathogenic microorganisms in various food matrices as exemplified by bacteria Campylobacter Jejuni, Escherichia Coli, Listeria monocytogenes, and Salmonella Typhimurium, is the major cause of most foodborne diseases globally. Whenever there is a foodborne pathogenic microorganism outbreak, product recalls or bulk destroying will be adopted as risk management measures to safeguard public health, which will incur substantial losses to local economy, international trade, and consumer confidence consequently.

DNA aptamers are developed against surface proteins of Campylobacter Jejuni (Bruno, 2009). The assay, which could be evaluated using both heat-killed and live Campylobacter Jejuni, is based on a magnetic bead (MB) with quantum dot (QD) sandwich assay scheme, and exhibits detection limits as low as an average of 2.5 colony forming unit (CFU) equivalents in buffer and 10–250 CFU in different food matrices.

DNA aptamer EcO 4R against Escherichia Coli 8739 outer membrane protein (OMP) with the sequence of 5’-ATCCGTCACA CCTGCTCTAC GGCGCTCCCA ACAGGCCTCT CCTTACGGCA TATTATGGTG TTGGCTCCCG TAT-3’ is recently identified using a fluorescence resonance energy transfer (FRET) screening system, a detection sensitivity of as few as 30 live unlabeled Escherichia Coli per ml is achieved in a competitive FRET assay

successfully (Bruno, 2010).

(Figure 3)

DNA aptamer with the sequence of 5'-ATC CAT GGG GCG GAG ATG AGG GGG AGG AGG GCG GGT ACC CGG TTG AT-3' to surface protein of Listeria monocytogenes is isolated as the reporter element sandwiched with anti-Listeria antibody for fiber-optic biosensor detection of Listeria monocytogenes (Ohk, 2010), the assay is able to selectively detect pathogenic Listeria in pure culture and in mixture with other bacteria at a concentration of approx. 10^3 CFU per ml, and could successfully detect Listeria monocytogenes from artificially contaminated meat products such as sliced beef, chicken and turkey after 18 h of enrichment.

For the detection of Salmonella Typhimurium, A total of 66 candidate sequences are enriched against Salmonella Typhimurium OMP, and the specificity is evaluated by gel-shift analysis for further aptamer selection, two aptamers with characteristic sequences of TATGGCGGCGTCACCCGACGGGGACTTGACATTATGACAG and GAGGAAAGTCTATAGCAGAGGAGATGTGTGAACCGAGTAA are eventually isolated, respectively. Real-time PCR detection sensitivity of 10 CFU per mL pure culture or per g fecal sample can be acquired when aptamer-conjugated magnetic beads are used for the capture of Salmonella Typhimurium (Joshi, 2009).

5.5. Biotoxin

Biotoxin, which might be categorized as mycotoxin, phytotoxin, neurotoxin and some other toxins derived from animal origin, is a toxic substance produced by a living organism. biotoxin can pose great hazard to people if existing in raw or processed foods.

Aptamer to ochratoxin A (OTA), a mycotoxin that widely occurs in wheat and other

foodstuffs, is firstly developed by Canadian scientist (Cruz-Aguado, 2008). The aptamer selected in this work with the sequence of GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA exhibits a dissociation constant in the nanomolar range, does not bind structural similar compounds such as N-acetylphenylalanine or warfarin, and binds with a 100-fold less affinity to ochratoxin B, The selected aptamer can be used for the determination OTA in wheat samples at ppb levels.

Another aptamer to Fumonisin B1, a mycotoxin produced by Fusarium verticillioides and F. proliferatum in corn, is recently developed using eighteen rounds of SELEX (Maureen, 2010). The aptamer with the sequence of AATCGCATTACCTTATACCAGCTTATTCAATTACGTCTGCACATACCAGCTTATTCAATT binds to Fumonisin B1 with a dissociation constant of 100 ± 30 nM is acquired to show the potential for use in biosensor-based detection.

Ricin, a phytotoxin naturally present in castor beans, is recently detected in orange juice and milk using a DNA aptamer-based surface-enhanced Raman scattering (SERS) scheme (Lili, 2011). Ricin B chain is first captured from food matrices by a thiolated aptamer conjugated silver dendrites and then SERS spectrum is collected for principal component analysis (PCA). Compared with antibody-based approaches, this detection scheme presents assay promptness, ease of manipulation, and improved sensitivity. The assay can be finished within 40 minutes, the limit of detection for ricin B chain is 50 ng mL in orange juice and 100 ng mL in milk, respectively.

5.6. Heavy metals

Heavy metals, as exemplified by arsenic, lead and mercury, when existing in

toxicologically effective levels, can deteriorate human physical and mental function, and damage human lungs, kidneys, liver and other vital organs, long-term exposure to heavy metals from diet or other routes may result in cancers or various slowly progressing skeletal, muscular, or neurological degenerative diseases.

A high affinity ssDNA aptamer that can bind to arsenite (As(III)) and arsenate ((As(V)) with dissociation constants of 7 and 5 nM respectively is developed (Kim Mina, 2009). Candidate aptamers are selected by conventional SELEX with a control scheme from a randomized DNA library (5'-GGT AAT ACG ACT CAC TAT AGG GAG ATA CCA GCT TAT TCA ATT-N40-AGA TAG TAA GTG CAA TCT-3'), aptamer affinities are measured using surface plasmon resonance (SPR), and aptamer specialty is successfully characterized with other nine metal cations.

For the detection of mercury ions (Hg^{2+}), a simple and sensitive ssDNA aptamer-based colorimetric detection method is developed (Li, 2009). The anti- Hg^{2+} aptamer is rich in thymine (T) and can readily form T-Hg^{2+}-T configuration, and the assay is based on the measuring of the color change induced by the presence of Hg^{2+}, which shows a linear response toward Hg^{2+} concentration of 1 x 10-4 mol L^{-1} to 1 x 10^{-9} mol L^{-1}, the spectrometric detection limit is 0.6 nM, and the aptamer shows excellent selectivity over other eleven metal cations.

Recently, a fluorescence technique for the detection of Pb^{2+} ions using polyguanine /terbium ions (Tb^{3+}) conjugate is developed as well (Lin, 2011). Pb^{2+} ions competes with Tb^{3+} ions to form complexes with polyguanine, the extent of formation of the polyguanine-Tb^{3+} complexes decreases upon increasing the Pb^{2+} concentration, leading to decreased fluorescence at 545 nm when excited at 290 nm. A two-step fluorescence measurement is used to minimize

interference from Hg^{2+}. The fluorescence signal obtained is linear with respect to the Pb^{2+} concentration over the range of 3.0-50 nM, and the limits of detection for Pb^{2+} ions is 1.0 nM.

5.7. Food allergen

Food allergens, which are mostly plant or animal proteins in foods, can trigger mild to severe allergic reactions including dermatitis, gastrointestinal dysfunction, respiratory distress, and even life-threatening anaphylaxis. The scope of food allergy, particularly for young people, is a potential health concern that needs to be addressed thereof.

Although it may vary regionally, the most common food allergens are milk, eggs, peanuts, tree nuts, seafood, shellfish, soy and wheat. Lysozyme, which is a dominant egg-white protein and is frequently used as an anti-inflammatory drug or natural antibacterial agent, can cause severe allergic reactions even when present in minute amount.

CE-SELEX is used to screen aptamers against egg-white lysozyme from a ssDNA library (Dinh, 2010), the collection time is chosen after 3.4 min of separation for aptamer-ssDNA complex from unbound DNA, only five rounds of selection are needed for the selecting eventually. The selected aptamer with the sequence of 5'-GCAGCTAAGCAGGCGGCTCACAAAACCATTCGCATGCGGC-3' has a dissociation constant of 2.8 ± 0.3 nM as determined by fluorescence anisotropy and confirmed with surface plasmon resonance measurement, the aptamer is successfully substantiated for its specificity against other egg white proteins (ovomucoid, ovotransferrin) and bovine serum albumin (BSA), this extraordinary work paves the way for the detection and quantification of lysozyme as allergen in foods to ensure compliance with product labeling and improve consumer protection.

(Figure 4)

5.8. Genetically modified organisms

Accompanied by the fast development of biotechnology and agro-business during the past two decades, genetically modified organisms (GMOs) have been claimed as a complementary solution to enhance crop productivity and secure food supply globally. However, some unpredictable adverse effects such as gene transferring, allergy, intoxication, carcinogenesis, and some observable antibiotic resistances, are all documented occasionally, which might attribute to GMOs. Because of the relevant difficulties for long term health risk assessment, the safety of foods derived from GMOs still remains unsubstantiated.

In order to develop exogenous protein characterizing method for GM foods safety assessment, researchers from Chinese inspection and quarantine regime (Shuxun, 2010) have used a custom synthesized random ssDNA library to screen aptamers against recombinant 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein from genetically modified maize by conventional SELEX with 16 rounds of selection. The selected aptamers are cloned and sequenced, primary structural analysis of sequence homology and secondary structural prediction are all performed to characterize aptamer-protein affinity, aptamer with the sequence of CCAGGGGGGC CCCTGGACCC CGCGCTCTAG AGATGGAGCG ACATGGACGC GCAACCCTCG ATG GGGTAGG is suggested as a trial for further detection method development.

5.9. Persistent organic pollutants

Persistent organic pollutants (POPs), as exemplified by dioxins and polychlorinated biphenyls (PCBs), are notorious industrial chemicals which can enter human food chain by environmental migration and cause severe food safety issues even at trace levels, POPs levels

surveillance is of utmost importance thereof.

Sponsored by US EPA, Eno River Labs, LLC propose to develop a dioxin testing kit using a fluorescently tagged ssDNA aptamers for selective binding and quantitative analysis of dioxin (Bharat, 2006), the project is initially focused on generating a set of specific fluorescent DNA aptamers in the presence of PCBs to bind 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the dioxin with the highest toxic equivalency factor (TEF). A synthesized 4-amino-2,3,7,8-TCDD is proved significantly less reactive to Sepharose resin to demonstrate the inability of the selected aptamer binding to 2,3,7,8-TCDD rather than the column, Eno River Labs, LLC has identified that aptamer selection process is relatively easier for moderate polar molecules with active functionalities that can be dissolved in an aqueous medium, while aptamer production is not optimized for non-polar, hydrophobic, and nonreactive molecules.

Currently, there are no aptamer based kits commercially available for dioxins or PCBs detection, however, as complementary schemes to existing high resolution mass spectrometry and antibody based screening methods, aptamer based assays are highly expected and will be developed for the determination of POPs in food matrixes in the near future.

5.10. Other Chemical Food Toxicants

Pesticides, which are the most widely used agro-chemicals globally especially in developing countries, have posed as great threatens for food safety and human health if pesticide residuals and relevant metabolites in food matrixes exceed their maximum tolerance levels. As an alternative to antibody for screening purpose, aptamer has ever emerged as a potential tool for pesticide detection. Aptamers targeting insecticide acetamiprid are selected by SELEX strategy after 18 rounds of repeated selection (Jiang, 2011), the ssDNA pool is enriched and then 14

sequences are selected and carefully identified, and an -specific aptamer with the apparent dissociation constant (Kd) estimated to be 4.98 μM is successfully obtained. Although the affinity of this ssDNA aptamer is somewhat lower than that of typical antibodies, the aptamer can be further used for the field determination of acetamiprid in relevant foods and/or other agricultural products.

(Figure 5)

Additionally, migrated chemicals from food packaging materials are of great health concern and should be characterized from various food matrixes nowadays. For instance, bisphenol A (BPA), an endocrine disruptor and a monomer migrant in the products of polycarbonate plastics, is recently detected by aptamer sandwich-based carbon nanotube field effect transistor (FET) sensor with 1 pM sensitivity and an even lower limit of detection of 10 fM using additional biotin modification on labeling aptamer (Joohyung, 2011), a random ssDNA library with a collection of the sequences 5'-GGGCCGTTCGAACACGAGCATG-N60-GGACAGTACTCAGGTCATCCTAGG-3' is chemically synthesized for aptamer selection, and this is the first successful demonstration of aptamer based CNT sensor for the detection of small molecules, which will be supposed to find its great potential for food safety applications in the future.

Furthermore, illegal food adulterant, such as melamine, which was intentionally added to dairy products to get faked apparent protein contents, is recently subjected to determination using an aptamer-based resonance scatting (RS) assay scheme (Zhiliang, 2011). nanosilver is modified by ssDNA aptamer to obtain a RS probe for melamine, based on the catalytic effect of the probe on the fehling particle reaction, a nanocatalytic RS is proposed for the determination of 0.02-1.06

μg L-1 melamine successfully.

A quick referential summary of above-mentioned aptamer and its potential applications for food safety, is tabulated in Table 3 concisely.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

Aptamers, which had so far been evolved for 20 years approximately, have great merits over antibodies in terms of detection ease and assay performance, some important SELEX variants with improved efficiency for aptamer discovery are well developed in recent years, and aforementioned aptamer applications have shown potential advantages for the detection of food pathogens practically.

However, the application of aptamers for food safety analysis is still in its infancy, reports of real world food pathogen detection are still limited, aptamers against some important food pathogens , which includes dioxin, norovius, aflatoxin, and some toxins of animal origin, are still not available. In addition, aptamer-based commercial detection kits for routine or ad hoc inspection are not procurable as well.

Further research will be focused on the screening of aptamers with great affinity and developing relevant assay kits for not only food pathogens not reviewed herein but also multi- or categorized food pathogens such as pesticides or veterinary drugs with structural or functional similarities. Investigating aptamer array- based schemes for high throughput detection, and discovering aptamers for regulatory management to target quality or geographic markers for food authenticity characterization, will be developed as highly expected, accordingly.

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Table 1. Aptamers versus antibodies

Table 2. Important SELEX Variants

Table 3. A quick referential overview of aptamer and its potential applications for food safety

Figure 1. Schematic SELEX protocol

Figure 2. Schematic presentation of dual-aptamer strategy for sensitive discrimination of prion disease-associated isoform. (Reprinted with permission from Anal. Chem. 2010□82□23□: 9738. Copyright 2010 American Chemical Society.)

Figure 3. Secondary structures of EcO 4R and EcO 5R aptamers by use of web-based Vienna RNA software using DNA parameters at 25 °C. Only loop 5 of EcO 4R labeled with Alexa Fluor (AF) 647 between bases 42 and 43 (from the 5' end) produced noteworthy FRET when reacted with ten-fold dilutions of unlabeled live E. coli 8739. (Reprinted with permission from J Fluoresc. 2010, 20, 1218. Copyright 2010 Springer.)

Figure 4. Choosing the collection window for CE-SELEX to screen anti-losozyme aptamer. (Reprinted with permission from Molecules, 2010, 15, 1129. Copyright 2010 MDPI Publishing.)

Figure 5. Specificity and affinity determination for anti-acetamiprid aptamer. (a) Specificity was analyzed by comparing the ability to signal the presence of different pesticides, and (b) affinity was detected by the binding assay. (Reprinted with permission from Journal of Agricultural and Food Chemistry. 2011, 59, 1585. Copyright 2011 American Chemical Society.)

Table 1

Characters	**Aptamers**	**Antibodies**
Selection environment	under a variety of conditions	Limited to physiologic conditions

Production process	Efficient with limited processes	Time consuming and costly
Batch activity	Uniform	Varies
Physical stability	Good	Bad
Shelf life	Prolonged	Limited
Targets applicable	Applicable for any targets	Difficult for toxins or non-immunogens
Binding affinity	in low nanomolar to picomolar range, and in micromolar scale for small molecules	in low nanomolar to picomolar range, and in micromolar scale for small molecules
Binding modifiability	Can be changed on demand	Difficult to adjust
Detection range	Better	Good
Target sites	Aptatopes can be determined by the investigator	Epitopes determined by animal immune system
Cross reactivity	Attainable	Not attainable
Function alterability	Wide variety of chemical modifications for diverse functions	Limited modifications applicable

Table 2

Category	Designation	Characteristics	Refs.
Rationalizing starting pools	Genomic SELEX or cDNA-SELEX	SELEX library is derived from a certain organism's whole genome (e.g., cDNA fragments), human, yeast and *E. Coli* genomes are reported for the construction of SELEX library. This scheme is advantageous for exploring the interaction of bioactive molecules and nuclear acids, and can further be utilized to study the regulation mechanism between ligands and nuclear acids as well.	Dobbelstein et al., 1995; Gold et al., 1997; Singer et al., 1997; Shimada et al., 2005; Kim et al., 2003

	Photo SELEX	Modified nucleotide activated by absorption of light is incorporated in place of a native base in either RNA- or in ssDNA-randomized oligonucleotide libraries, PhotoSELEX-evolved aptamers capable of simultaneous quantification of a large number of analyte molecules is feasible with the merits of improved binding or specificity.	Jensen et al., 1995; Golden et al., 2000
	Phosphoro(di)thioate SELEX	Replacing oxygen by sulfur in phosphodiester linkage which can result in non-specific interactions, producing a phosphoro(di)thioate linkage and thus have high nuclease resistance and can increase binding affinity without sacrificing specificity.	Jhaveri et al., 1998; Yang et al., 1999

	Covalent- SELEX or Cross-linking SELEX	The uracil analogs 5-iodouracil or 5-bromouracil can be incorporated into a randomized oligonucleotide pool for covalent linking to target molecules to provide better binding affinity and specificity.	Jensen et al., 1995; Kopylov et al., 2000
	Tailored SELEX or Primer-free SELEX	The schemes are often utilized to obtain short aptamers or spiegelmers largely free of the fixed nucleotide sequences of the initial libraries.	Vater et al., 2003; Wen et al., 2004; Pan et al., 2008
	Chimeric SELEX	Using two or more different libraries for production of chimerical aptamers with more than one desired feature or function. Each parent library is selected for a distinct feature, then the aptamers selected are fused.	Burke et al., 1998

	Multistage SELEX	Similar to chimeric SELEX, the scheme uses separate oligos obtained from different pools to select aptamers with the ability to bind dual targets, which are important for elucidating DNA allosteric mechanism and related diagnosis application.	Wu and Curran, 1999
improving aptamers' selectivity	Negative SELEX	A protocol to remove unwantedly adsorbed oligonucleotides from the pool by the matrixes used for immobilizing targets to screen aptamers of higher affinity and selectivity.	Ellington, 1992
	Counter SELEX or Subtractive SELEX	Applying a selection step to related targets for the enrichment of oligonucleotide pool, which are not able to distinguish between structurally similar structures. Can generate aptamers of high specificity.	Jenison et al., 1994; Wang et al., 2003

	Deconvolution SELEX	The strategy facilitates rapid isolation of ligands to targets of special interest within the mixture of complex targets, and might provide a means for dissecting complicated biological systems.	Morris, 1998
Enhancing targets applicability.	Complex target SELEX	To generate aptamer when a pure target is hard to obtain, nonessential, or the research object itself is inherently complicated targets focused. The differences between complex targets SELEX and normal SELEX lies in the constitution of the targets.	Shamah et al., 2008
	TECS-SELEX	Target expressed on cell surface SELEX. Natural or recombinant target proteins are expressed on the surface of a certain cell, then aptamers are selected by complex targets SELEX accordingly.	Ohuchi et al., 2006

	Toggle SELEX	Target-switching SELEX to deal with several kinds of targets simultaneously, can facilitate aptamer functional diversification and bypass tedious protein purification steps.	Radrizzani 1999; White et al., 2001; Bianchini et al., 2001
	Blended SELEX	A SELEX variant that other molecules, which can lead the oligonuclear acid chain to the specific region of a target, are mixed to form a blended screening pool to get high pertinent aptamers.	Smith et al., 1995; Hamm 2002
	Expressions cassette SELEX or SELEX-SAGE	Special forms of blended SELEX with transcription factors being blended.	Martell et al., 2002
	Mirror-image SELEX or Spiegelmer Technology	Screen aptamers for enantiomer target from dextrorotatory oligonucleotide pool, then synthesize resulting aptamers as levorotatory oligonucleotide Spiegelmers with superior binding and stability because L-isomers will not be recognized by ribonuclease.	Eulberg et al., 2003; Faulhammer et al., 2004

	Whole Cell-SELEX	Cell-based selection with counterselection strategy to collect DNA sequences that interact only with the target cells	Shangguan et al., 2006
	Whole Bacteria - SELEX	A singular aptamer that binds to virulent strain with high affinity and specificity was identified for antimycobacterial application using whole bacteria as the target.	Fan et al., 2007
Attaining higher efficiecy	Mono-SELEX	Starts with one affinity chromatography to sort non-binding oligonucleotides, low-affinity aptamers and high-affinity aptamers. Highly affine aptamers are amplified once and characterized further by an aptamer blot assay.	Nitsche et al., 2007
	Non-SELEX or NECEEM-SELEX	A repetitive partitioning process with no amplification needed. Using nonequilibrium capillary electrophoresis of equilibrium mixtures (NECEEM) for partitioning.	Berezovski et al., 2006

	CE-SELEX	Using capillary electrophoresis for partitioning to enhance the efficiency for aptamer selecting.	Mendonsa and Bowser, 2004; Mosing et al., 2005
	Microfluidic SELEX	Using miniaturized microfluidic platform for high efficient partitioning and aptamer selection	Hybarger, 2006; Mosing, 2007; Lou, 2009
	Automated SELEX or HTS-SELEX	SELEX protocol automation or high throughput(HTS) screening feasible based on SELEX workstation	Cox, 1998; Drolet, 1999; Eulberg, 2005
	FluMag-SELEX	Target immobilized on magnetic beads for aptamer selection. DNA oligonucleotides modified with fluorescein.	Stoltenburg, 2005
Other	*In silico* SELEX	Using computational molecular docking or virtual screening strategy to screen aptamers. Need downstream real experiments to validate preliminary *in silico* results.	Yaroslav, 2009; Xuemei, 2011

Table 3

Category	Targets	Sample matrices	Detection methods
Acute pathogens	Prion protein (PrP) isoform of transmissible spongiform encephalopathies (TSEs)	Fetal bovine serum and mice brain homogenate	Fluorescence emission
	Specific Avian influenza virus (AIV) H5N1 gene sequence	PBS buffer	Differential pulse voltammetry (DPV)
Dyes / Fungicides	Malachite Green/ Leucomalachite Green	Fish tissue	Fluorescence spectrophotometry
	4,4'-Methylenedianiline	Environmental or biological samples	Colorimetric or chromatographic methods
Antibiotics	Chloramphenicol	N/A	N/A
	Tetracycline	PBS buffer and milk	Electrochemical detection
	Oxytetracycline (OTC)	Tris-based buffer	Electrochemical detection
	Streptomycin	N/A	N/A

	Tobramycin	Human serum	Faradaic-electrochemical impedance spectroscopy
	Tobramycin, kanamycin and neomycin	N/A	N/A
Microorganisms	Surface protein of Campylobacter Jejuni	Tris-based buffer , beef extract, chicken juice, and 2% milk	Fluorometric Detection
	Escherichia Coli 8739 outer membrane protein	Tris-based buffer	Fluorescence resonance energy transfer (FRET) assay
	Surface protein of Listeria monocytogenes	Pure culture, bacteria mixture, artificially contaminated beef, chicken and turkey	Fibre-optic biosensor
	Salmonella Typhimurium outer membrane protein	Chicken sample	Real-time PCR
Biotoxin	Ochratoxin A (OTA)	Wheat grain	Fluorometric Detection

	Fumonisin B1	N/A	N/A
	Ricin	Orange juice, milk	Surface-enhanced Raman scattering (SERS) detection
Heavy metals	As^{3+} / As^{5+}	Lab prepared solution and water sample	Inductively coupled plasma mass spectrometry (ICP-MS)
	Hg^{2+}	Aqueous media	Colorimetric detection
	Pb^{2+}	Soil and water	Fluorescence spectrometry
Food allergen	Egg-white lysozyme	N/A	N/A
Genetically modified organisms	Maize 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) protein	N/A	N/A
Persistent organic pollutants (POPs)	PCBs	N/A*	N/A*
	Dioxin (TCDD)	N/A*	N/A*
Other Food Toxicants	Insecticide acetamiprid	N/A	N/A

Toxicants	Migrant bisphenol A (BPA)	Tris-based buffer	Carbon nanotube-based field effect transistor sensor
	Adulterant melamine	Pre-treated milk	Resonance scattering

N/A: Application not available but expectable.

N/A*: Both aptamer and application are not available.

Figure 1

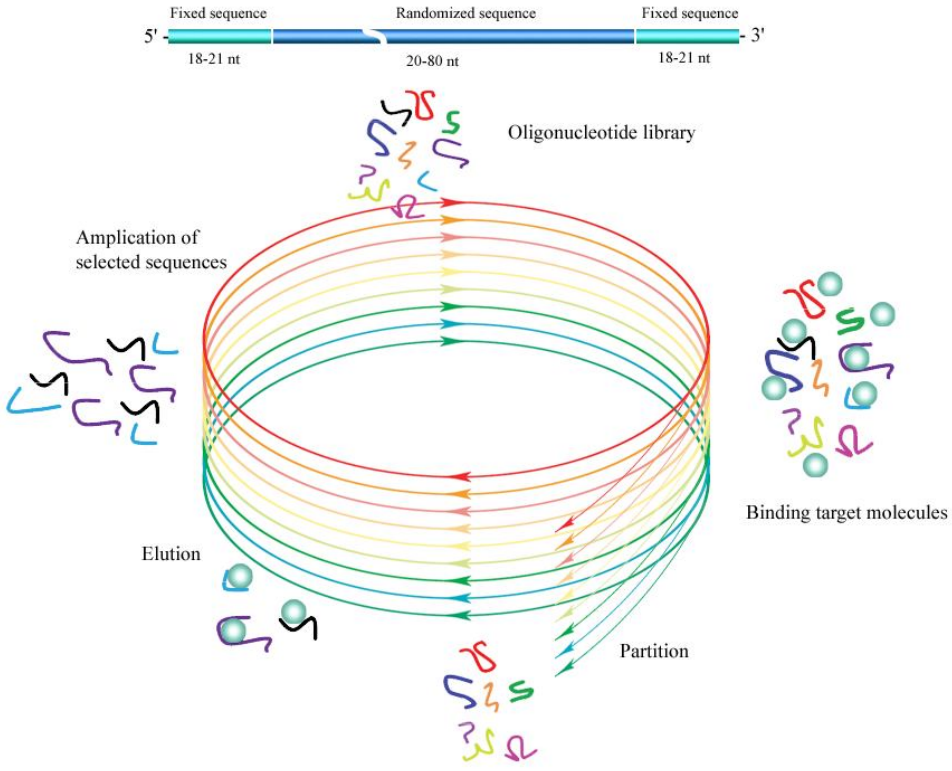

Figure 2

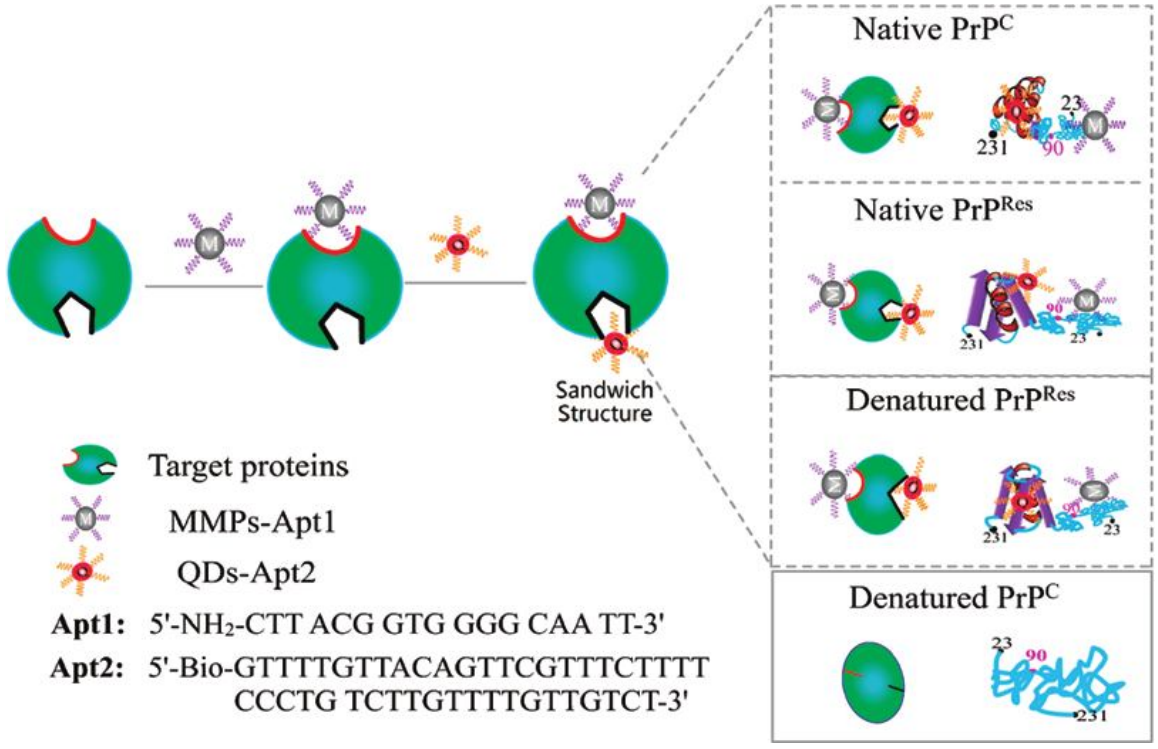

Figure 3

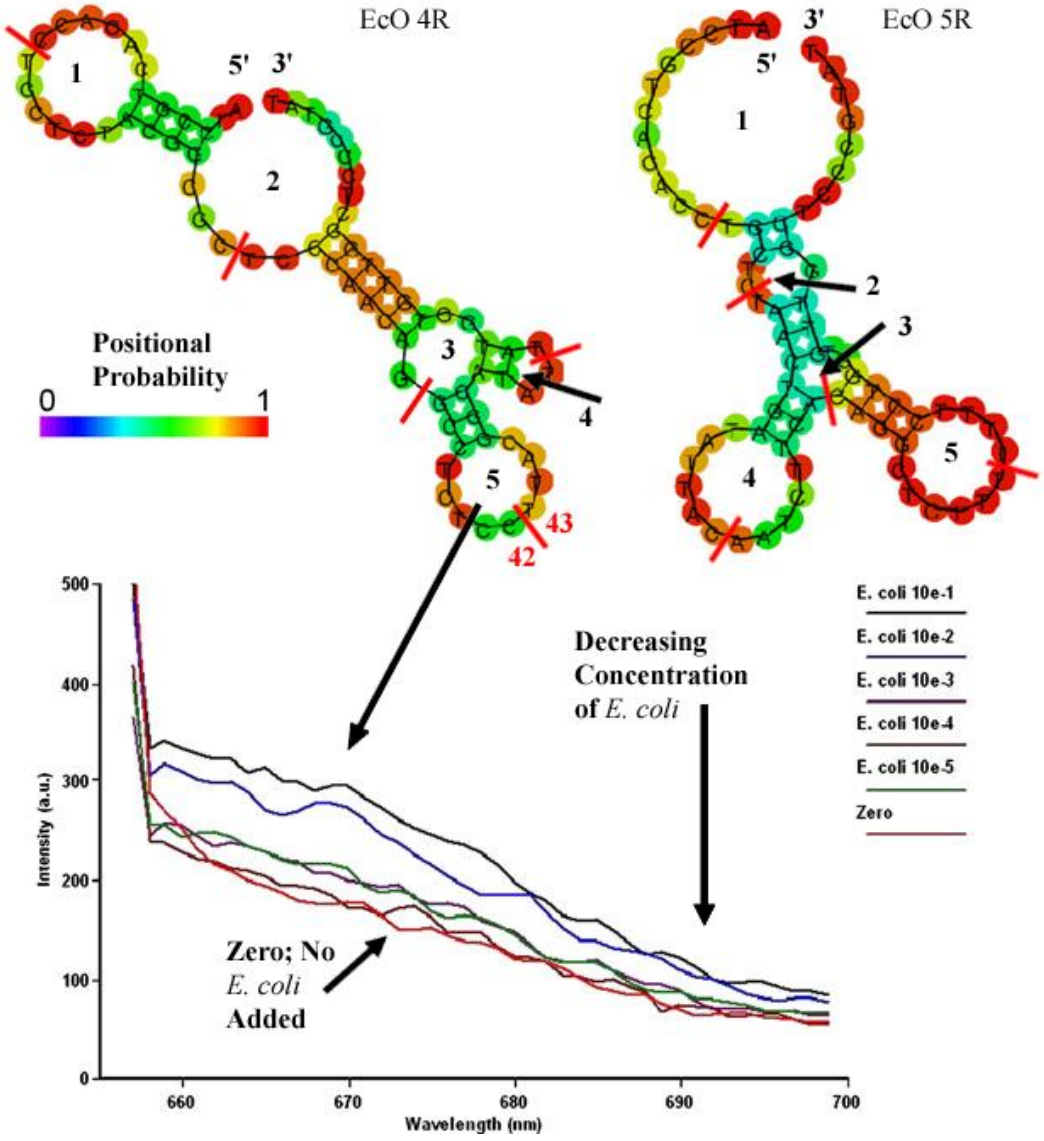

Figure 4

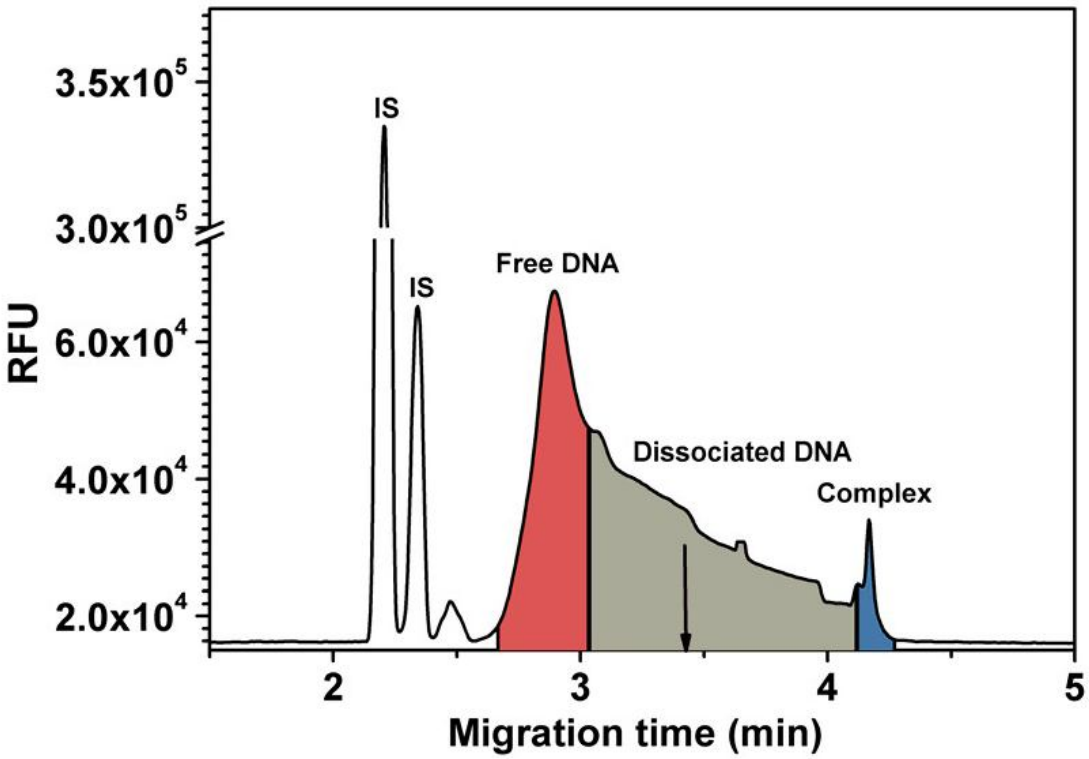

Figure 5