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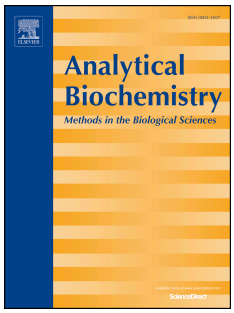

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Abdullah Tahir Bayraç, Sultan Ilayda Donmez

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Selection of DNA Aptamers to *Streptococcus pneumonia* and fabrication of graphene oxide based fluorescent assay

Abdullah Tahir BAYRAÇ[1], Sultan Ilayda DONMEZ[1]

[1]Department of Bioengineering, Karamanoglu Mehmetbey University, Yunus Emre Campus, 70100

Karaman, Turkey

bayrac@kmu.edu.tr

Abstract

Pneumococci are one of the leading causes of infections throughout the world causing problems mainly in children, elderly, and immune-deficient patients. In recent years antibiotic resistant *Streptococcus pneumoniae* strains become widespread. Therefore simple, rapid, and specific detection methods are needed for public health. In this study, DNA aptamer probes against *S. pneumoniae* were selected using bacterial Systematic Evolution of Ligands by Exponential Enrichment (SELEX) and these probes were integrated in to a graphene oxide (GO) based fluorescent assay. Among the tested aptamers three candidates Lyd-1, Lyd-2 and Lyd-3 showed K_d values of 844.7±123.6, 1984.8±347.5, and 661.8±111.3 nM, respectively. These candidates showed binding affinity to *S. pneumoniae* and no specific binding to the bacteria used in negative selection. The binding of aptamers were showed by fluorescence spectroscopy and flow cytometry. GO based label-free fluorescent assay developed using Lyd-3 aptamer had a unique detection limit of 15 cfu.mL^{-1}. Thus we believe that the selected aptamers and fabricated GO based assay has potential to be used in the detection of *S. pneumoniae*. Selected aptamers selectively bind to *S. pneumonia* with anti-pneumococcal potential and holds great potential to be used as molecular probes for identifying and targeting.

Key words: Aptamer; *pneumoniae*; SELEX; pathogen biosensor; biofilm

INTRODUCTION

S. pneumoniae is an encapsulated Gram-positive, catalase-negative, diplococcus aerotolerant anaerobe bacterium often found in the human nasopharyngeal microbiota [1,2]. Although it is a part of the normal flora, *S. pneumoniae* is a pathogenic bacterium and one of the major causes of pneumoniae, otitis media, meningitis, and bacteraemia [3,4]. The World Health Organization (WHO) estimated ~1 million deaths of children from pneumonia, under 5 years old in 2015[5]. *S. pneumoniae* is the most frequent source of community-acquired bacterial pneumonia in adults[6]. Since penicillin was very effective against *S. pneumoniae* and the side effects are more tolerable than other antibiotics, it has been widely used and the resistance of *S. pneumoniae* to antibiotics was first noted in 1977 [7]. Antibiotic resistant *S. pneumoniae* strains are now a worldwide problem with a need of new classes of antibiotics [8].

Biofilms are layers of bacterial populations that attach to each other and to tissues or inert surfaces such as medical equipment[9]. Attachment of these bacterial populations are generally sustained by polysaccharide, protein, and nucleic acid based polymeric matrix [10]. This matrix strengthens the defense of bacteria to the host immune system and increases the tolerance to antibiotics [9,11]. More than 65% of the bacterial infections are caused by biofilm forming bacteria[8,12]. Pneumococcal biofilms can form in the sinus mucosa [13], adenoid and mucosal epithelium[4] and involved in otitis media and cystic fibrosis [14]. Furthermore *S. pneumonia* biofilms can cause clinical problems on many medical devices such as typanostomy tubes and cochlear implants [14].

Aptamers are single-stranded oligonucleotides that can bind to their target with high specificity, selectivity and affinity because of their 3-D shape[15–19]. Aptamers were discovered by 3 different research group independently in 1990 [20–22] and have been generated against a wide variety of targets such as drugs[23–25], proteins[26], organic or inorganic molecules[27], spores[28], bacteria[16,29,30], eukaryotic cells[15,31,32], eggs[33], and tissues[34]. Aptamers are nucleic acid analogues of antibodies and are generally 25 to 100 bases long[35]. Aptamers are generated by the SELEX (systematic evolution

of ligands by exponential enrichment) process in which sequences that has affinity to the target are enriched from a large population[36,37]. Shortly, SELEX is the repeating cycles of affinity separation and PCR enrichment of oligonucleotides from a starting pool. Cell-SELEX is a modified conventional SELEX that uses whole cells as a target. In Cell-SELEX all surface molecules on the cell are potential target for the selection procedure and this makes Cell-SELEX highly complex. Cell-SELEX is advantageous to traditional SELEX when the target need to be detected in it is physiological form or the marker target is unknown[38]. In the literature, there are some cell-SELEX and traditional SELEX derived aptamers for pathogenic bacteria such as *Staphylococcus aureus*[39,40], *Salmonella spp*[16], *E.coli*[41], *Listeria monocytogenes*[42], *Mycobacterium tuberculosis*[43], *Streptococcus mutans*[44], *Streptococcus pyogenes*[45] etc. Metagenomics studies showed that there are many types of bacteria in the human body. Only gastrointestinal microbiome has more than 40.000 bacterial species[46] and most of these bacteria are not characterized[47]. Also, there are many pathogenic bacteria that cause infections in human body and the majority of these bacteria have not had aptamers selected specifically yet. In this paper, we developed aptamers that can detect and also inhibit biofilm formation of *S. pneumoniae*.

Graphene oxide (GO) is a nanomaterial broadly used for developing biosensors because of its unique mechanical, electrical, optical and thermal properties [48]. GO is a single atom thick, hydrophilic, two dimensional graphitic carbon nanomaterial and aptamers can be physically adsorbed on to it easily by π-π stacking, hydrophobic interaction, hydrogen bonding, and van der Waals forces [49]. More importantly GO can efficiently quench various fluorophores by non-radioactive electronic excitation energy transfer [50]. High quenching efficiency of GO makes it a suitable material to be used with fluorophore tagged aptamers. There are many GO based bacterial biosensors in the literature using the quenching feature of the GO [51–55]. To the best of our knowledge this is the first report in selecting aptamer probes for *S. pneumoniae* using cell-SELEX, representing their anti-biofilm activities and using these aptamers in GO based fluorescence assay.

MATERIALS AND METHODS

Bacterial Strains and Culture Media

For *in vitro* selection, glycerol stocks of *S. pneumoniae* bacteria (Klein) Chester (ATCC® 49619™) and *S. pyogenes* (ATCC® 19615™) were streaked out onto brain heart infusion (BHI) agar plates and incubated anaerobically at 37°C using Merck Anaerocult® A System. After overnight anaerobic incubation, a single healthy colony was inoculated in to BHI broth supplemented with 5% heat inactivated fetal calf serum and grown to mid-log phase at 37°C without shaking in anaerobic condition. *Enterecoccus faecalis* (ATCC® 19433™) were cultured aerobically in the same culture media and conditions.

Primers and Library

The single stranded DNA libraries used in the study have 42 central random region flanked by two primers (Library1; 5'TGACGAGCCCAAGTTACCT(N42)AGAATCTCCGCTGCCTACA'3, Library1 forward primer; 5'FAM-TGACGAGCCCAAGTTACCT'3, Library1 reverse primer; 5'Biotin-TGTAGGCAGCGGAGATTCT-3, Library2; 5'AAGGGCTGGCTGGGATGGA(N42)TCACTTCACGG ACCCCACT'3, Library2 forward primer; 5'FAM-AAGGGCTGGCTGGATGGA'3 Library2 reverse primer; 5'Biotin-AGTGGGGTCCGTGGAGTGA-3). DNA libraries and primers were purchased from IDT DNA Technologies.

Cell-SELEX Protocol

Whole alive bacterial cells were used in all SELEX experiments. Two different SELEX procedures were performed throughout the study. In the first one, 10 cycles of SELEX were carried out only against *S. pneumoniae* (Klein) Chester (ATCC® 49619™) whereas in the second one, 20 cycles of SELEX were carried out against *S. pneumoniae* bacteria (Klein) Chester (ATCC® 49619™) with the negative selection against *Streptococcus pyogenes* (ATCC® 19615™) and *E. faecalis* (ATCC® 19433™). The schematic representation of SELEX was summarized in Figure S1. Briefly, for SELEX, 5 nmol of ssDNA library was dissolved in 300 µL of binding buffer (4.5 g/L glucose, 5 mM $MgCl_2$, 1 mg/mL BSA in Dulbecco's

PBS with $CaCl_2$ and $MgCl_2$ (Sigma D1283), pH 7.2). The library was incubated for 5 min at 95°C for denaturation and cooled down on ice until incubation with bacteria. All bacteria used in SELEX experiments were cultured until optical density at 600 nm wavelength (OD_{600}) reached to 0.3, centrifuged at 3200 g for 5 min at 37°C and washed 2 times using 1000 µL of binding buffer. After washing step solution was centrifuged again to pellet down the bacteria and supernatant was removed from the tube. Then, 300 µL of denaturated ssDNA library was incubated immediately with $1.0x10^8$ CFU of bacteria pellet for 60 min using Tube Revolver/Rotator (Thermo, 88881001) at 15 rpm. After incubation, cells were centrifuged and the pellet was washed using 1000 µL of washing buffer (4.5 g/L glucose, 5 mM $MgCl_2$ in Dulbecco's PBS with $CaCl_2$ and $MgCl_2$ (Sigma D1283), pH 7.2). To separate the bound ssDNA from target the pellet was resuspended in 500 µL DNase free water and incubated at 95°C for 5 min. The eluted sequences were amplified using PCR with 6-Carboxyfluorescein (FAM) and biotin labeled primers. PCR was performed using Biorad C1000 Touch™ Thermal Cycler and contained 2 µL of template ssDNA, 1 µL of each primer (10 µM), 2 µL of 10X PCR Buffer, 1.6 µL of dNTP mix(2.5 mM), 4 µL $MgCl_2$ (25 mM), Taq polymerase (5 U/ µL) and 9.34 µL of DNase free water (denaturation 94°C for 30 sec, annealing at 59°C for 30 sec, extension at 72°C for 30 sec). For each round of SELEX a preparative PCR was carried for optimization of PCR cycle number. The highest number of PCR cycle without any nonspecific band in the agarose gel electrophoresis was chosen and 1 mL of PCR was carried out in aliquots of 50 µL using this cycle number. After amplification, double stranded PCR product was immobilized on streptavidin-coated Sepharose (GE Healthcare Bio-Sciences Corp.) with the aid of biotin labeled primer and FAM labelled ssDNA was separated by alkaline denaturation using 500 µL of 200 mM NaOH for 15 min. Nap-5 column (GE Healthcare Bio-Sciences Corp.) was used to remove NaOH from the ssDNA solution. After SELEX round one, 100 pmol of ssDNA pool was used for each selection, incubation time was reduced from 60 to 30 min and washing was increased from 1 to 3 times gradually as the number of selection round increased. Negative selection using *Streptococcus pyogenes* and *Enterococcus faecalis* was introduced to selection scheme in the 7^{th} and 9^{th} rounds of SELEX to increase the selectivity of aptamers.

Sequencing and Bioinformatics

For sequencing Illumina 16S metagenomic sequencing library preparation procedure was modified using suitable library primer sequences instead of V3 and V4 regions. After the final SELEX round, pools of 8^{th} , 9^{th}, and 10^{th} from first SELEX and 4^{th}, 6^{th}, 8^{th}, 10^{th}, 12^{th}, 14^{th},16^{th},18^{th}, and 20^{th} from the second SELEX with negative selection were multiplied using illimuna overhang adapter sequence added SELEX primers; 5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG□[SELEX F primer]3' , 5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG□[SELEX R primer]3'. After confirmation of PCR products with agarose gel electrophoresis, they were purified using AMPure XP beads. After purification, each round of SELEX was dual indexed using Nextera XT Index primers. After PCR clean up using AMPure XP beads library quantification, normalization, pooling, and denaturation were carried out according to the manufacturer's instructions. Sequencing was accomplished using 150 cycle MiSeq Reagent Kit v3 with the addition of 10% adapter ligated PhiX control.

Sequences retrieved from each pool were firstly analyzed using NextGen Sequence Workbench v3.2.3; low quality reads and reads out of the range of 79 to 81 bases long were removed from the pools. Avalanche Sequence Dereplicator was used to be able to find the most frequent and enriched sequences. Most frequent 20 sequences were chosen for further analysis. The primer regions were removed from the sequences and they were aligned using MAFFT v7 software[56]. The alignments were visualized using Jalview 2.10.2[57] with respect to clustalX and base similarity. Phylogenetic tree of aligned sequences was build using unweighted pair group method with arithmetic mean using ATV 4.0 [58]. After forming the phylogenetic tree, representative sequences from different families were selected as candidate aptamers. These candidate aptamers' 3-D shapes at 4°C and 37°C were constituted using NUPACK [59] and the sequences preserving their shapes at higher temperatures were chosen and synthesized for further experiments.

Determination of Dissociation Constants

The binding affinities of aptamers were determined by incubating various concentrations of FAM labeled sequences with $1x10^8$ cells at 4°C for 30 min. After incubation cells were washed twice with 500 µL

washing buffer and mean fluorescence intensity was determined using fluorescence spectrophotometry. The ligand binding analysis function of SigmaPlot was used to calculate the apparent K_d of aptamers according to the equation $Y=(B_{max}X)/(K_d+X)$ (where Y is the net fluorescence intensity at each concentration, the B_{max} is the saturated binding and the X is the concentration of aptamer in nM).

Specificity of Selected Aptamers

Specificities of the aptamers were determined by using fluorescence spectrophotometry, flow cytometry, and fluorescence microscopy. For specificity experiments *S. pneumoniae, S. pyogenes,* and *E. faecalis* were used as target cells. Also, a scrambled 80bp DNA sequence was used as a control DNA sequence. For specificity experiments $1x10^7$ $cfu.mL^{-1}$ of cells were incubated with 250 nM FAM labeled aptamer and control sequence for 15 minutes than washed twice with 500 µL washing buffer. The cells with bound aptamers were analyzed using flow cytometry (BD Accuri, C6 Plus) and fluorescence spectrophotometry (Hitachi, F-2700).

Effects on biofilm formation

Biofilm formation was quantified by modified crystal violet (CV) assay [60,61]. Briefly, *S. pneumonia* cells were cultured until OD_{600} reached to 0.3. Then, 100 µL of bacteria were inoculated in a 96-well plate and aptamers with a final concentration of 200, 400, 600, 800 and 1000 nM were added to assigned wells. Cells were cultured anaerobically for 12 hours and 1% crystal violet was added to each well. After 15 minute of incubation with crystal violet cells were removed and wells are washed with saline solution. In order to detach the biofilm from plate walls, 400 µL of 96% ethanol was added and solution was transferred to a new tube. After addition of 600 µL of sterile distilled water, each sample's absorbance was determined at 595 nm (maximum absorption wavelength of CV).

Graphene oxide based label-free fluorescent assay

In order to integrate a selected aptamer to a GO platform 250 nM of Lyd-3 aptamer was used in fluorescent assay. For the GO concentration optimization, 250 nM of aptamer was titrated against 0-250 µg/mL GO (Hazerfen Corp.) and Stern-Volmer plot was generated for the optimization of quenching. For

the detection of *S. pneumoniae* different numbers of bacteria (10^1 to 10^7 cfu/mL) were incubated with the GO/aptamer solution for 30 min at room temperature and fluorescence spectra were recorded using fluorescence spectrophotometer (Excitation wavelength 495 nm, Emission wavelength 520 nm). Selectivity of the GO/aptamer platform was tested using 10^7 cfu/mL *S. pneumoniae* and $2x10^8$ cfu/mL *S. pyogenes, E. faecalis, E. coli* and *S. aureus* in washing buffer.

Statistical analysis

Results were expressed as mean± standard deviation. Unless otherwise stated, all experiments were performed in triplicate. The dissociation rates and the constants of the aptamer–target complexes were determined using SigmaPlot 11.0.

RESULTS AND DISCUSSION

Aptamer Selection

Whole Cell Bacterium SELEX was used in this study to generate aptamers that specifically bind to *S. pneumoniae* cells. Using whole bacterium as a target in a SELEX provides an aptamer selection for surface entities in their native and working conformations. *S. pyogenes* and *E. faecalis* were used for negative targeting to increase the selectivity of the developed aptamers. While choosing these negative targets phylogenetical proximities were in the nearest focus, so they thought to be in the same order of *Lactobacillales*. Also, by choosing these two bacteria we planned to generate aptamers that differentiates α-hemolytic bacteria (*S. pneumoniae*) from β-hemolytic (*S. pyogenes*) and γ-hemolytic (*E. faecalis*) bacteria. Besides, in order to specifically inhibit the biofilm formation of *S. pneumoniae* sortase homology was taken into consideration which has an important role in the colonization and pilus assembly[62].

The enrichment of the pools generated throughout the SELEX was monitored by fluorescence pool binding assay (Figure S2). The results of this assay showed that after SELEX round 16, no further

increase in fluorescence was observable which indicated the saturation of the selection. Throughout the selection process, stringency was increased with the decrease in incubation time and application of extra washing steps. This pressure applied in the selection resulted in the enrichment of highly specific sequences through the last rounds.

Therefore, SELEX process was stopped at round 20 and pools were sequenced for the determination of aptamer candidates. Approximately, 0.8 billion high quality score base (~10 million 80 bp aptamer sequence) sequence results were obtained after sequencing and basic filtering of the results. After designating the most frequent 20 sequences, phylogenetic tree and the Multiple Alignment using Fast Fourier Transform (MAFFT) were formed in order to find the families. Most frequent member of the each family was chosen and their structural motifs were analyzed at 4°C and 37°C. Since they preserved their structural motifs such as hairpins and bulges at higher temperatures due to their high GC content, Lyd-1, Lyd-2 and Lyd-3 were chosen as candidate aptamers for further characterization studies (Figure 1).

Characterization and binding studies of selected aptamers

Aptamers were tested for their binding abilities to target cells by fluorescence spectroscopy using FAM labeled sequences. As shown in Figure 3, aptamers showed binding affinities with equilibrium dissociation constants in the submicromolar range. Lyd-3 showed the highest binding affinity with a K_d of 661.8±11.3 nM. Lyd-1 and Lyd-2 had a K_d value of 844.7±123.6 nM and 1984.8±347.5 nM respectively (Figure 2). These aptamers were also tested with other bacteria to determine the specificity for molecular recognition of *S. pneumoniae*. Even *S. pyogenes* and *E. faecalis* which are in the same order and phylogenetically very close to *S. pneumoniae* didn't show a discernable binding compared to the target cells (Figure 3). Although the binding study of Lyd-2 with target cell showed approximately the same fluorescence intensity with that of Lyd-3, it had higher binding affinity towards non-target cells as compared to Lyd-3. Based on these results, it was concluded that Lyd-3 was the best differentiating aptamer with higher fluorescence difference between target and other cells. Binding efficiencies of the aptamers were also measured using flow cytometry and compared to a scrambled 80 bp sequence as demonstrated in Figure 4. While there was not a shift for target bacteria with control DNA sequence, the

binding of aptamers were much more intense showing distinct shifts in logarithmic scale. The three aptamer sequences with high affinity and specificity were generated as a result.

Effect of the aptamer on biofilm formation

Microorganisms living in natural environment generally form clusters on surfaces by residing in the extracellular matrix surrounding cells. The matrix produced by these bacteria prevents them to be in direct contact with the environmental factors such as antibacterial agents, metabolites and immune system factors and the biomass comprised of this matrix and cells are called biofilm. Many cell surface proteins are involved in the initial attachment to surfaces, thereby facilitating the colonization to form biofilm architectures. Virtually, all wild-type pneumococci are able to form a biofilm[63] and many surface proteins are taking role in the initial attachment[64]. We screened our aptamers for their effect on biofilm formation and found that Lyd-3 aptamer had the best anti-biofilm activity. When incubated with 100 nM of Lyd-1 and Lyd-2, biofilm formation decreased to 90.8% and 92.6% respectively, while the Lyd-3 caused a decrease to 67.2% with respect to no aptamer control. Incubation of *S. pneumoniae* with increasing concentrations of Lyd-3 suppressed the biofilm formation significantly. Biofilm formation was reduced from 100% to 35.8% at 1µM of Lyd-3 concentration with respect to no aptamer control (Figure 5). These results demonstrated that the biofilm forming ability of *S. pneumoniae* was significantly impaired by the application of Lyd-3 aptamer. *S. pneumoniae* forms biofilms during nasopharyngeal colonization; the former which facilitates persistence, the latter, a prerequisite for subsequent development of invasive disease [64]. Previous colonization events causing biofilm formation explains the *S. pneumoniae* susceptible individuals. Aptamers that are capable of preventing biofilm formation can be a potential solution to colonization of bacteria when used with suitable antibiotics.

Graphene oxide based label-free fluorescent assay

Since Lyd-3 showed the best selectivity and an anti-biofilm activity we have chosen it for the GO assay. Combination of GO and aptamers has high potential to develop aptasensors because of the high

performance adsorption of ssDNA on GO by π-π stacking [65]. Moreover, GO quenches many types of fluorescent molecules due to intrasheet energy or electron transfer [66]. In this assay FAM labeled Lyd-3 aptamer was adsorbed on the surface of GO and this caused the quenching of fluorescence signal of the aptamer. The Stern-Volmer plot displayed a sigmoidal curving shape in the range of 0 to 250 $\mu g.mL^{-1}$ (Figure 6A) with a characteristic feature of the combination of dynamic and static quenching [67]. Maximum quenching of the fluorescence was reached by 200 $\mu g/mL^{-1}$ GO with 90 % and further increases of the GO concentration didn't caused a decrease in the fluorescence. Therefore 200 $\mu g.mL^{-1}$ GO and 250 nM Lyd-3 aptamer was selected as optimum for further experiments. As shown in Figure 6B and c the fluorescence intensity of the system increased with the addition of increasing number of target cells. Increase in the fluorescence with increasing numbers of target cells was caused by the split up of the Lyd-3 aptamer from the GO and binding to target molecules on the cell. The limit of the detection (LOD) of the assay was calculated as 15 cfu/mL using the the $3\sigma/S$ formula where σ is the standart deviation of the blank and S is the slope (Figure 6C). There are many methods reported for the detection of *S.pneumoniae* like PCR [68], single tube loop mediated isothermal amplification [69], q-PCR [70], nested PCR [71], multiplex PCR [72] and electrochemical methods [5]. Analytical parameters of these methods have been compared in Table S1 and it can be seen that our method has one of the widest linear range and comparable LOD. The selectivity of the GO-Lyd-3 assay was investigated using *S.pneumoniae* and other bacteria. Although all other bacteria species showed a slight increase in fluorescence it is not significant compared to target cells (Figure 6D). Nonspecific interactions such as ionic interaction and ligand adsorption between cell surface and ssDNA are thought to be causing this background fluorescence. Nevertheless, GO-Lyd-3 assay showed more than 3 times fluorescence increase with respect to the bacteria with the highest background.

CONCLUSIONS

In the present work, we successfully identified aptamers that specifically recognized *S.pneumoniae* via cell-SELEX and showed that these aptamers can be used in the detection using fluorescence spectroscopy and flow cytometry. Three highly specific aptamers generated for *S. pneumoniae*, Lyd-1, Lyd-2 and Lyd-

3, were developed using cell-SELEX with K_d of 0.84 µM, 1.98 µM, and 0,66 µM respectively. These generated aptamers showed higher specificities toward their target cell by differentiating non-target but closely related bacteria. In addition to their higher affinities and specificities, aptamers were evaluated to determine their anti-biofilm activity and it was shown that Lyd-3 successfully inhibited the biofilm formation of *S.pneumoniae* by up to 35.8%. Also a GO based fluorescent assay developed using Lyd-3 aptamer showed a LOD of 15 cfu.mL^{-1}. All these results suggested that aptamers developed in this study might be potential probes for further research in the discovery of proteins in charge of *S. pneumoniae* biofilm initiation. To the best of our knowledge, we are the first to report the generation of aptamers and aptamer mediated detection of live *S. pneumonia* cells and demonstrate their anti-biofilm activity.

Conflict of interest

The authors declared that they have no commercial or financial conflicts of interest.

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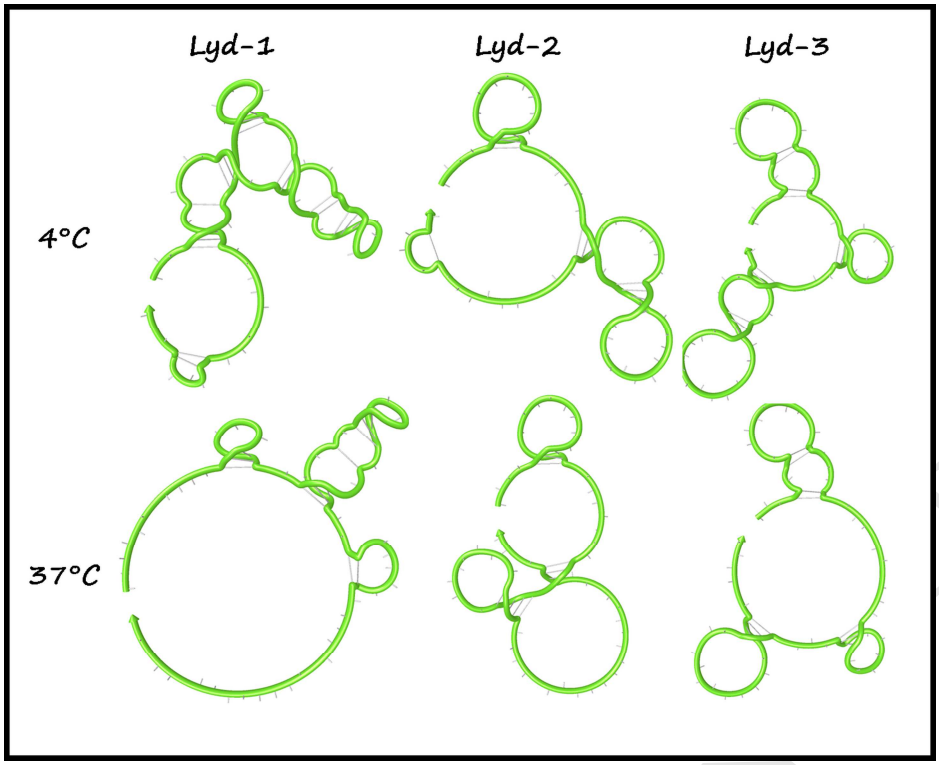
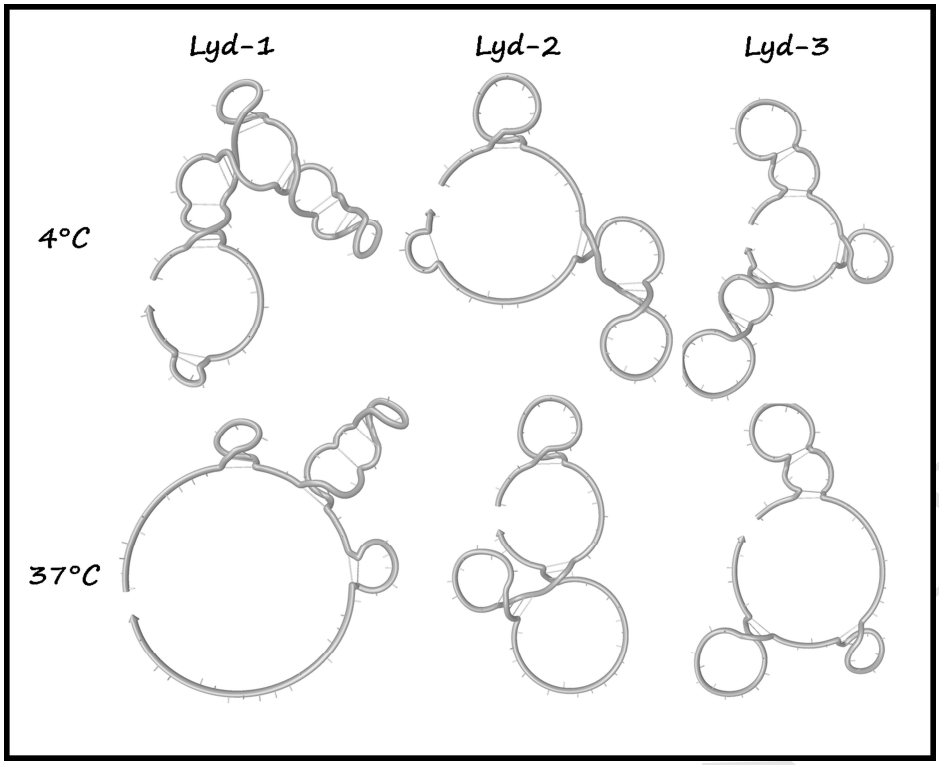
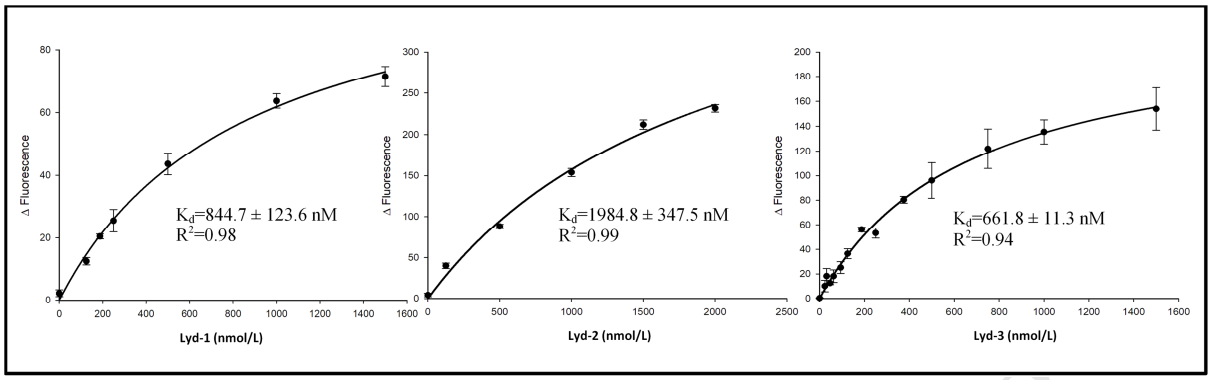
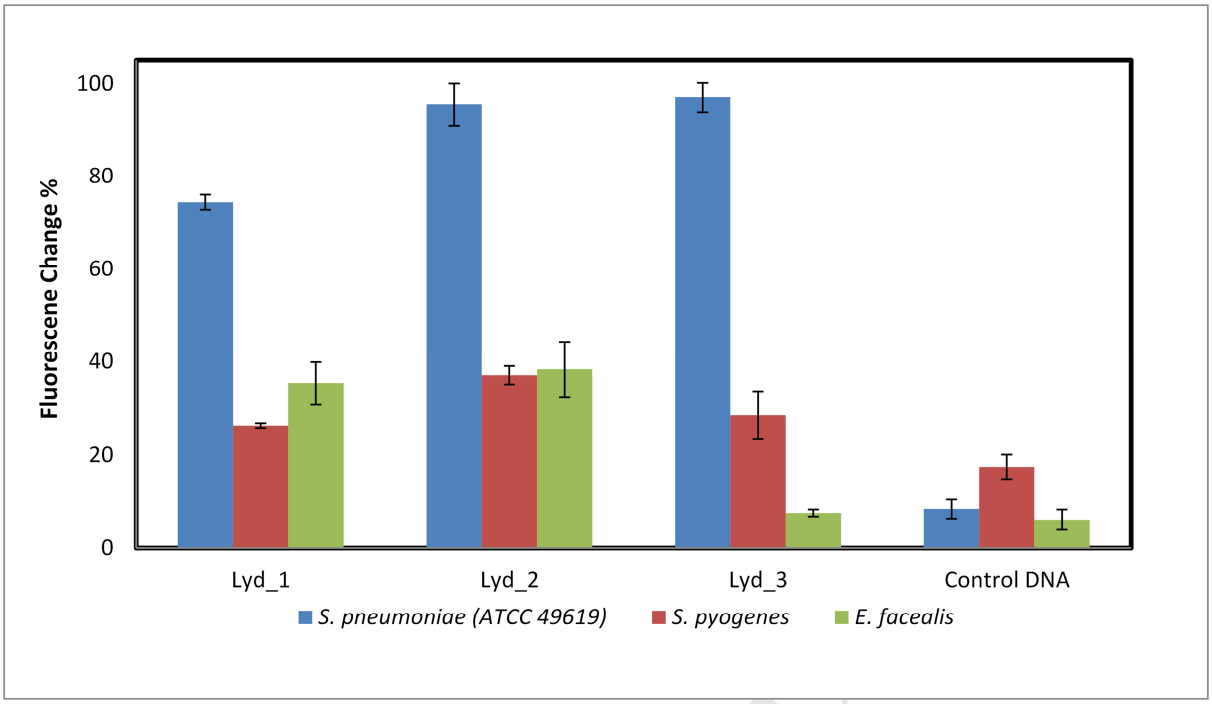
Table 1 Sequences and Binding Affinities of Selected Aptamers

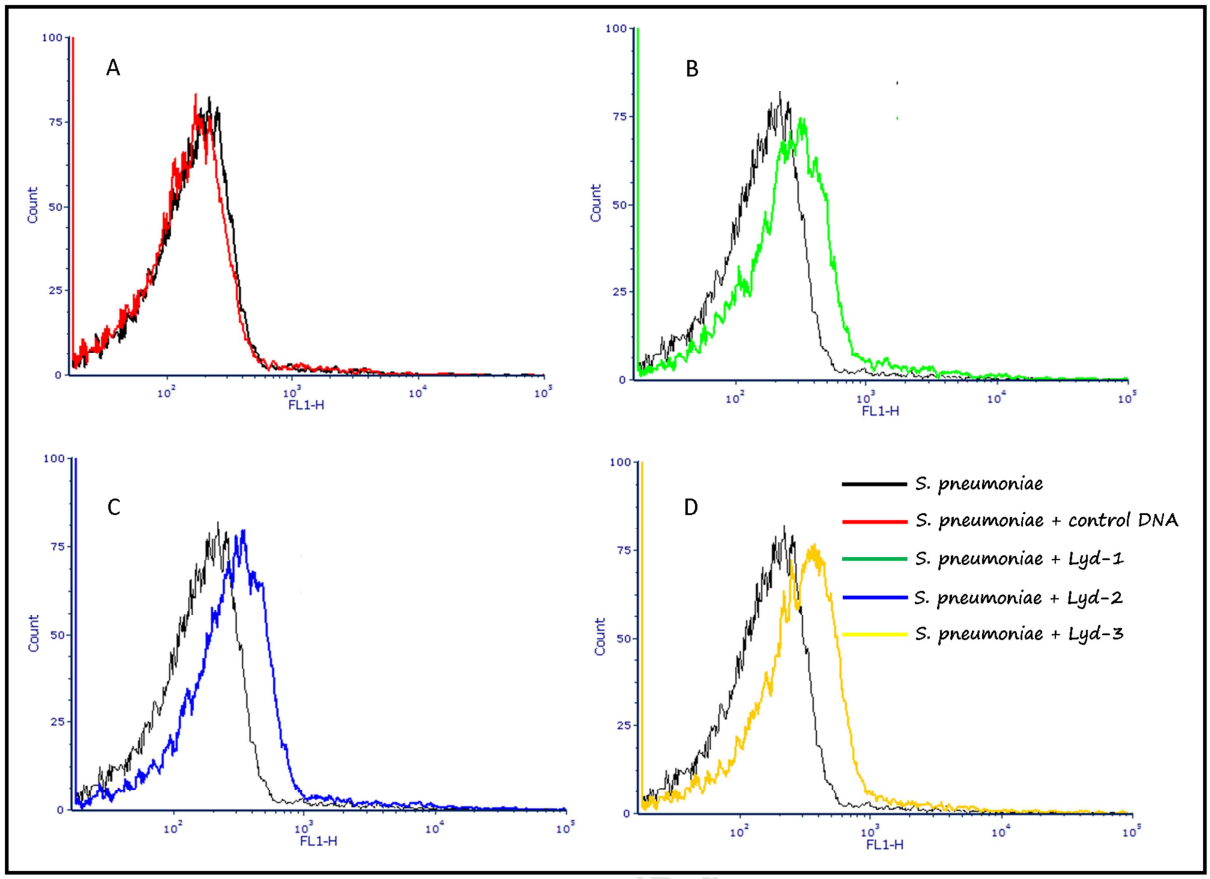
Name of the Aptamer	**Sequence of the Aptamer**	**K_d Values (nM)**
Lyd-1	AAGGGCTGGCTGGGATGGACCCTCCCGAAACGAGCTGTCT CTTAACGGAAGCTAATCTGCCTCACTCCACGGACCCCACT	844.7±123.6
Lyd-2	TGACGAGCCCAAGTTACCTCACCCGCCTGGCAAAAAACACC ACGACATTTTTCTCACCCCCAGAATCTCCGCTGCCTACA	1984.8±347.5
Lyd-3	TGACGAGCCCAAGTTACCTGCCCCCGAACCATACCACACGA TGCCCCGTACCCCAGCCACCAGAATCTCCGCTGCCTACA	661.8±111.3

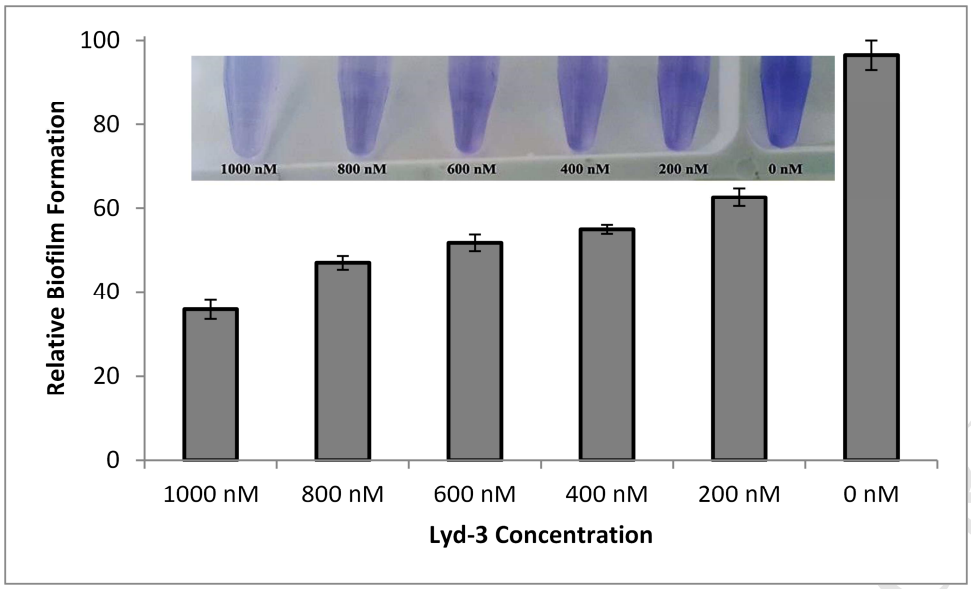


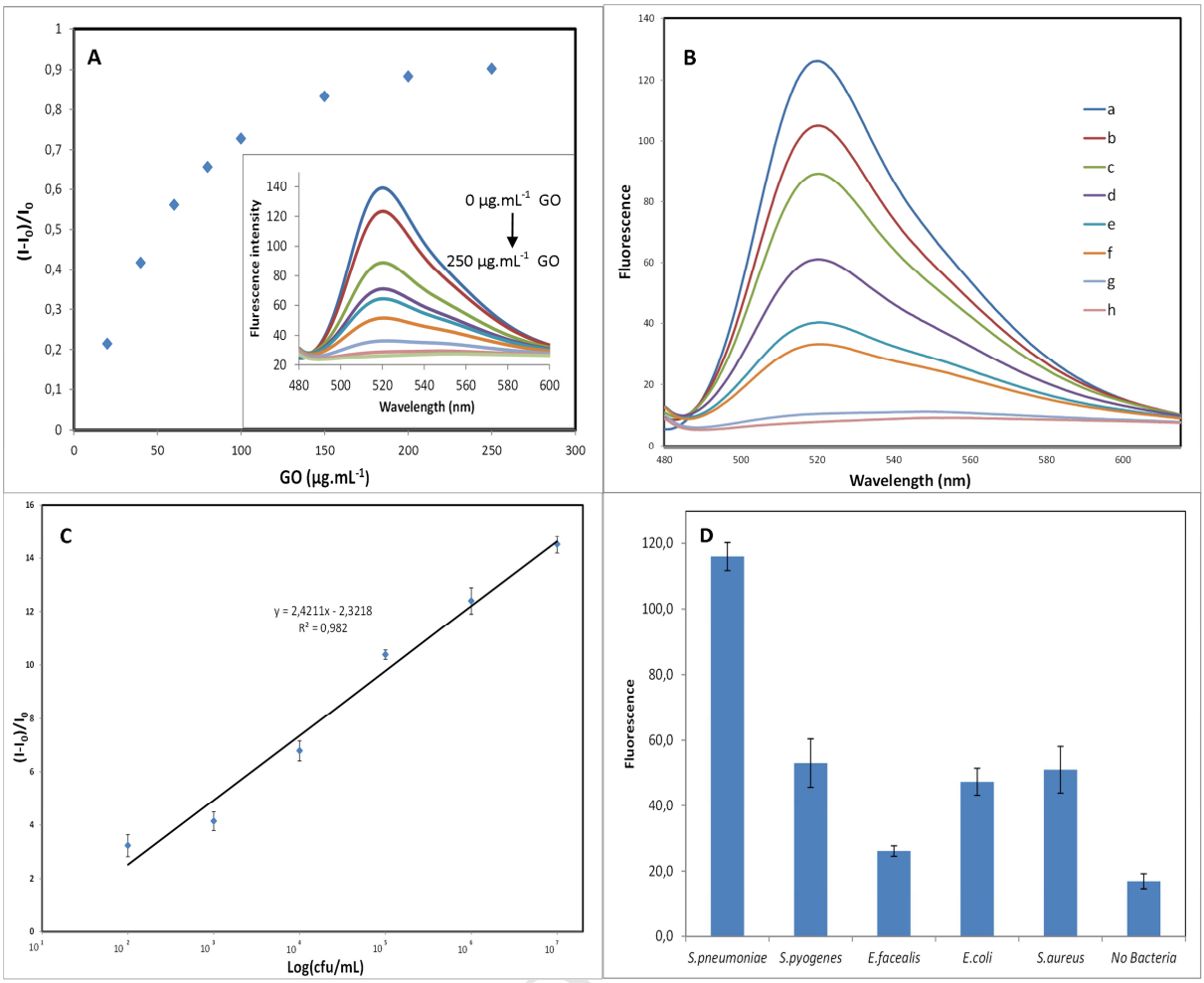












Highlights

Selection of DNA Aptamers specific to *Streptococcus pneumonia* and fabrication of graphene oxide based fluorescent assay

Abdullah Tahir BAYRAÇ[1], Sultan Ilayda DONMEZ[1]

- ssDNA aptamers were selected against *Streptococcus pneumoniae* by using bacterial SELEX.
- Graphene oxide/aptamer based label-free fluorescent assay was constructed to detect *Streptococcus pneumoniae*.
- The limit of detection of the sensor was 15 cfu.mL^{-1} of *S. pneumoniae*.
- Lyd-3 aptamer showed inhibition on biofilm formation.