

Label-free G-Quadruplex aptamer and Thioflavin-T based turn-off fluorescent detection of ethanolamine

Abdullah Tahir Bayraç*, Yasemin Acar

Department of Bioengineering, Karamanoğlu Mehmetbey University, 70100, Karaman, Turkey

ARTICLE INFO

Keywords:

Ethanolamine
Aptasensor
G-quadruplex
Fluorescence molecular rotor

ABSTRACT

A label free aptasensor based on G-quadruplex forming ethanolamine aptamers and Thioflavin T was developed for the selective and sensitive detection of ethanolamine. The detection system consists of DNA aptamers with four GGG repeats forming a G-quadruplex that Thioflavin T can easily bind to causing high fluorescence emission intensity because of its fluorescence molecular rotor nature. In the presence of ethanolamine, binding of aptamer to its target induces conformational changes causing the separation of Thioflavin T from the complex with a dramatic decrease in fluorescence intensity of reaction mixture. This fluorescence aptasensor has a convenient sensitivity and selectivity with a detection limit of 641 μM to measure ethanolamine directly in contaminated water samples in a wide linear range. Furthermore this method avoids all complicated modification and labelling steps and thus offers a simple, fast, and cost efficient solution for ethanolamine detection.

1. Introduction

Ethanolamine (2-aminoethanol) is a viscous and hygroscopic amino alcohol having molar mass of 61.08 g mol^{-1} . It is extensively used in textile, dry cleaning, rubber manufacturing and pharmaceutical industry, for the production of dyes, adhesives, pesticides, insecticides, medicines, surfactants, and gas scrubbers [1]. Ethanolamine is an irritant chemical for skin and eyes and also can cause severe problems for nervous and respiratory systems. Ethanolamine is associated with many diseases such as Alzheimer [2], atherosclerosis [3], hyperlipemia [4], schizophrenia [5], major depressive disorder [6] and ethanolaminuria [7]. It is found to be toxic to most organisms [8], acute oral median lethal dose (LD50) is 700 mg kg^{-1} for mice and 1000 mg kg^{-1} for rabbit [9]. Also median lethal concentration (LC50) ranges from 150 to 2100 mg L^{-1} for fish species. Because of its toxicity ethanolamine has to be monitored in industrial waste, groundwater and subsurface environments [4,11].

Detection of small molecules such as ethanolamine is a challenging and laborious for standard analytical methods [12]. Although various methods are studied in the literature [1,9,11,13,14] determination of the concentration of ethanolamine with both high accuracy and sensitivity is a compelling since it has a high polarity and low molecular weight which makes it hard to retain and separate from other substances in a matrix [4]. Hence, development of alternative methods for ethanolamine detection is of increasing importance. Recently, aptamers

become a hot subject in analytical chemistry because of their advantages such as high affinity, stability, flexibility, and modifiability with respect to antibodies [15]. Aptamers are single stranded oligonucleotide sequences, enriched and selected from a random oligonucleotide library by a technique named Systematic Evolution of Ligands by Exponential Enrichment (SELEX) and can bind to their target with high specificity and selectivity [16–18]. Many specific aptamers were selected for a wide range of targets such as small molecules [19,20], proteins [21], spores [22], bacteria [23–25], eukaryotic cells [26–29], eggs [30], and even tissues [31]. Aptamer mediated ethanolamine detection can eliminate the specificity problems of antibody based detection of small molecules. Ethanolamine aptamers were generated by a SELEX method [32,33] and many truncated variants were developed in order to find sequence motifs responsible for target binding [13]. Although the first ethanolamine aptamer was selected in 2005 by Mann et al., only an aptamer microarray [13] and an LC/MS aided indirect aptamer sensor [4] were developed up to date. Ethanolamine aptamers generated by Mann et al. have guanine rich nucleic acid sequences and can fold into G-quadruplex structures. G-quadruplexes are formed by the stacking of coplanar guanine tetrads via Hoogsten hydrogen bonds [34,35]. Coplanar architecture of G-quadruplexes has noticeable van der Waals attraction and many aromatic planar ligands can interact with this guanine chamber [36,37]. Thioflavin T(ThT) is one of the unique G-quadruplex ligand composed of benzothiazole (BZT) and dimethylaminobenzene (DMAB). ThT has been used as a fluorescence

* Corresponding author. Karamanoglu Mehmetbey University, Faculty of Engineering, Department of Bioengineering, 70100, Karaman, Turkey.
E-mail address: bayrac@kmu.edu.tr (A.T. Bayraç).

<https://doi.org/10.1016/j.dyepig.2019.107788>

Received 20 March 2019; Received in revised form 9 August 2019; Accepted 10 August 2019

Available online 11 August 2019

0143-7208/ © 2019 Elsevier Ltd. All rights reserved.

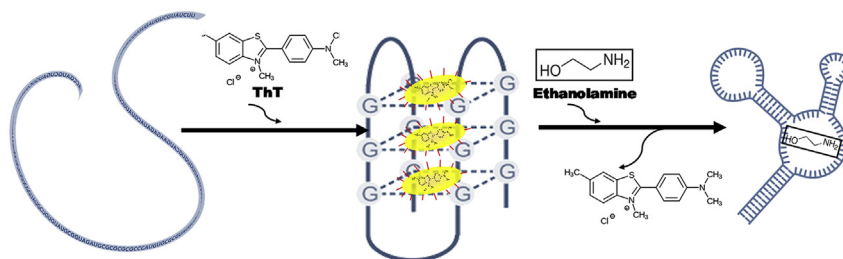


Fig. 1. Illustration of the label free ethanolamine aptasensor based on G-quadruplex formation.

reporter to quantify fibrils for more than 50 years which is thought to be found in protein-misfolding diseases such as Alzheimer and Parkinson [38]. When ThT is in a free state the dihedral angle rotated by the C–C bond between the BZT and DMAB moieties is 90° and molecule has the lowest quantum yield possible [39]. As ThT is embedded into the G-quadruplex its hidden fluorescence property will emerge with the suppression of steric hindrance [38–40]. So that aptamers with G-quadruplex sequences are good candidates for designing ThT based turn-off sensors. When aptamers with G-quadruplex sequences were incubated with ThT complex, they will show a high fluorescence yield with the insertion of the ThT in to the guanine cage, but after addition of the aptamer's target in to the environment aptamer will prefer to bind to its target and ThT will be unleashed and loose its fluorescence property (Fig. 1). Herein we report a label free G-quadruplex and ThT based aptasensor for the rapid and quantitative measurement of ethanolamine.

2. Experimental section

2.1. Chemicals, materials and measurements

Ethanolamine aptamers EA#9.4K46 5'-ATA CCA GCT TAT TCA ATT GCT GCG AGG TGG GTG GGT GGG AGC AAT T-3', EA#14.3K42 5'-ATA CCA GCT TAT TCA ATT TGA GGC GGG TGG GTG GGT TGA ATA-3', EA-ConsT 5'-GAG GTG GGT GGG TGG G-3' were synthesized and HPLC purified by Integrated DNA Technologies Inc. (Coralville, USA). Thioflavin T, ethanolamine, diethanolamine, methylene bis(acrylamide), 3-aminophenol, methanol, ethanol, isopropanol, glucose, glycerol, furfural, tris-HCl, sodium chloride, potassium chloride, magnesium chloride, calcium chloride, tween 20 were purchased from Sigma Aldrich and used without further purification. All chemicals were of analytical-reagent grade. Ultra-pure water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$) was used during all of the experiments. Binding Buffer (BB) (100 mM NaCl, 20 mM Tris-HCl, 5 mM KCl, 2 mM CaCl₂, 0.02% Tween 20, pH 7.6) was used for fluorescence detection of ethanolamine sensor.

All fluorescence measurement were carried out on an F7100 (Hitachi, Japan) with an excitation of 425 nm and an emission range from 450 to 600 nm at 1 nm increments. The slit widths for excitation and emission were set at 10 nm. The maximum emission intensity for ThT was observed at 495 nm. The pH was measured by inoLab pH 7110 and all experiments were performed at room temperature.

2.2. Feasibility test of the method

Feasibility of the method is tested with the usage of EA#14.3K42 ethanolamine aptamer. Four samples were prepared for the feasibility of the test; Sample 1 contained ThT only, Sample 2 contained ThT and ethanolamine, Sample 3 contained ThT and EA#14.3K42 aptamer and Sample 4 contained ThT, EA#14.3K42 aptamer and ethanolamine. All samples were prepared in BB and samples were incubated in room temperature for 15 min before taking measurements. For the image analyses of the test, Gel Doc™ XR + Gel Documentation System (BIO-RAD) was used.

2.3. Optimization of ethanolamine detection conditions

In order to get optimal results incubation time, concentration of ThT, concentration of aptamers, and concentration of KCl were optimized for each aptamer sequences. Aptamer concentration was in the range of 0–500 nM, ThT concentration was in the range of 0–11 μM, KCl concentration was in the range of 0–30 mM, and incubation time range was in the range of 0–60 min.

2.4. Sensitivity and selectivity of the assay

The ethanolamine detection was carried out in determined optimum conditions. Fluorescence change after the addition of different concentrations of ethanolamine was recorded and linear range, limit of detection (LOD), sensitivity and selectivity were determined accordingly. Selectivity of the sensor was tested using 60 mM of ethanolamine, diethanolamine, and 200 mM of methylenebis(acrylamide), methanol, ethanol, isopropanol, glucose, glycerol and furfural. All measurements were repeated for a minimum of three times with ten replicates for each sample.

3. Results and discussion

3.1. Design strategy

The designed strategy of the proposed method is explained in Fig. 1. Guanine rich ethanolamine aptamer can form a G-quadruplex complex with ThT. The fluorescence quantum yield of ThT increases upon embedding in to G-quadruplex because of its fluorescence molecular rotor (FMR) nature. But in the presence of ethanolamine, aptamer will prefer to bind to its target with its unique three dimensional shapes which will distort the G-quadruplex basis of the sequence, so that ThT molecules that leave the G-quadruplex complex will cause a dramatic decrease in the fluorescence intensity.

3.2. Feasibility test for the detection of ethanolamine

The feasibility of the developed method for ethanolamine detection was estimated by using EA#14.3K42 ethanolamine aptamer. In Fig. 2 it can be seen that only ThT showed a weak fluorescence intensity (Line A), even in the presence of 2 M ethanolamine (Line B). When ethanolamine aptamer and ThT were incubated together formation of G-quadruplex-ThT complex induced high fluorescence intensity (Line C). Upon addition of ethanolamine a dramatic decrease in fluorescence intensity, confirmed that the proposed method have ability to detect ethanolamine (Line D). Therefore, a label-free, easy, rapid but efficient ethanolamine detection method was established.

3.3. Optimization of sensing conditions

In order to get the highest fluorescence signal change, incubation time, ThT concentration, aptamer concentration, and K⁺ concentration was optimized using three different ethanolamine aptamer sequences. In all aptamers fluorescence intensity increased linearly with an

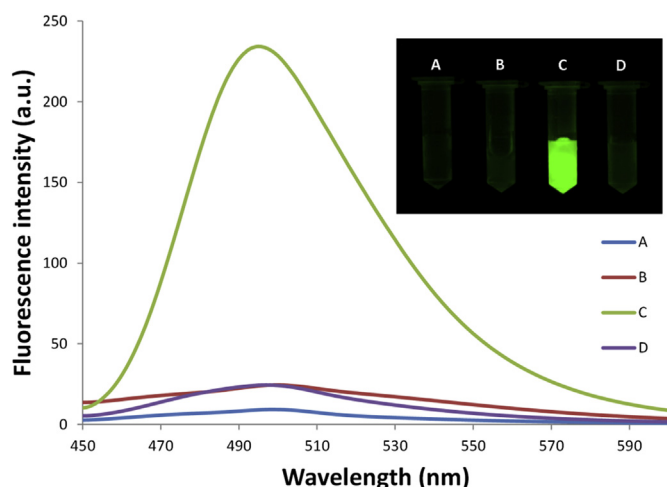


Fig. 2. Feasibility of the method. (A) Only 5 μM ThT, (B) 5 μM ThT and 2 M ethanolamine, (C) 5 μM ThT and 200 nM Aptamer, (D) 5 μM ThT, 2 M ethanolamine and 200 nM Aptamer. The inset is an image of samples under suitable excitation.

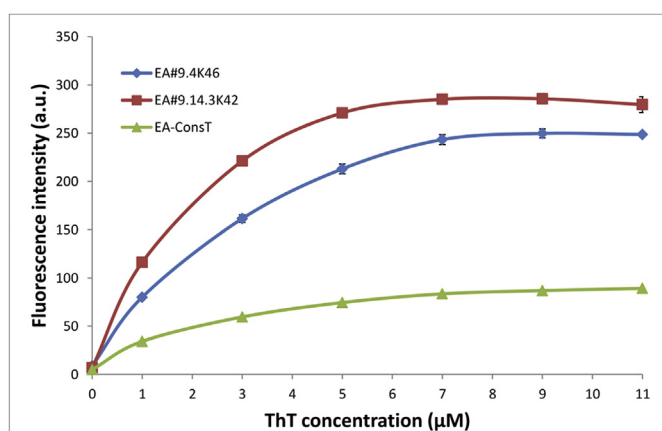


Fig. 3. Effect of the concentration of ThT on the fluorescence intensity for different aptamer sequences (200 nM).

increase in aptamer concentration (Fig S1A). ThT concentration was optimized for 200 nM aptamer and Fig. 3 shows that the fluorescence intensity enhanced as the ThT concentration was raised, reaching the maximum at 5 μM . Also it can be seen that the maximum fluorescence signal can be obtained from EA#14.3K42 aptamer. EA#9.4K46 (46 bp) and EA#14.3K42 (42bp) have similar curve forms and approximate fluorescence enhancement capacities for ThT but EA-Const showed lower fluorescence intensity enhancement. Although all aptamers had the same G-quadruplex motifs in their sequences the difference in the fluorescence intensity can be elucidated with the duplex connection at one end. Duplex connected G-quadruplex structures have more evident promotion on the fluorescent emission of turn-on dyes [41]. EA#9.4K46 and EA#14.3K42 aptamers has a duplex forming DNA sequences attached to their G-quadruplex forming motifs (Fig. S2) but EA-Const has only the G-quadruplex motif sequences so that the rigid duplex structure present in the EA#9.4K46 and EA#14.3K42 created more resistant for the rotational deactivation causing higher fluorescence property. Also K^+ concentration is very important for the stability of the G-quadruplex [42] and K^+ promotes the binding of ThT to G-quadruplex sequences [43]. Thus, 5 μM ThT with 200 nM EA#14.3K42 aptamer was used throughout the experiments in a BB which have 5 mM of KCl with an incubation time of 15 min.

3.4. Sensitivity, detection limit, linear range and repeatability of the assay for ethanolamine

In order to evaluate the sensitivity of the proposed assay, different concentrations of ethanolamine from 0 to 400 mM were added to system under optimal conditions. As shown in Fig. 4A the fluorescence intensity at 490 nm decreased with an increasing concentration of ethanolamine for EA#14.3K42. The decrease in the fluorescence intensity was continued up to 100 mM of ethanolamine and then stayed with background fluorescence (Fig. 4B). The inset of Fig. 4B shows that the fluorescence intensity had linear relationship with concentration in the range of 0.5 mM–75 mM with a correlation coefficient of 0.9945 for EA#14.3K42, 0.9892 for EA#9.4K46 and 0.9939 for EA-Const. EA#14.3K42 had the regression equation of $y = -3.0351x + 313.8$ with the lowest detection limit of 641 μM based on three times the signal-to-noise level compared to other two aptamers. Ten replicate measurements of 5 mM of ethanolamine showed the relative deviation of 0.33% which provides an excellent repeatability. Although there are many studies that have lower sensitivity for ethanolamine in the literature, the widest linear range is in our study (Table 1). Linear range is an important characteristic for the sensor performance and suitability of the linear range is a fundamental feature for direct measurements of targets in specific environments. To exemplify LC50 of ethanolamine for different fish species is in the range of 2.4 mM–34 mM [10] and also according to a microbial toxicity study, 50 mM of ethanolamine can inhibit bacterial growth up to 40% at pH 7.6 causing microbial deterioration in the environment which is a very common case in metal working industries in processes like grinding and drilling [44]. So that, it is important to have an assay for ethanolamine in a wide micromolar linear range and with an acceptable sensitivity in order to directly measure the ethanolamine in fresh water samples with high repeatability.

3.5. Specificity of the assay

To demonstrate the selectivity of the proposed assay, a series of small molecules were investigated by this method. As shown in Fig. 5 none of these molecules trigger a significant decrease in fluorescence intensity. The results indicate good selectivity of the presented sensing assay for the detection of ethanolamine.

3.6. Application

To evaluate the applicability of the assay for practical operation, sensor was applied to the detection of ethanolamine in artificially contaminated water samples and artificially contaminated water samples with 5% fetal bovine serum addition to see the matrix affect. The results showed that the recoveries were in the range of 95.5%–109.6% indicating the sensor was reliable and applicable for water samples. Addition of fetal bovine serum to mimic complex biological environment caused an increase in the recovery percentages but they were still in the acceptable ranges (Table 2).

Aptamers with G-quadruplex forming sequences were used in turn-off fluorescence sensors using dyes with fluorescence molecular motor property in the literature. Wu et al. used same strategy to detect ochratoxin a with better detection limit [45]. Zeng et al. also used ThT and G-quadruplex aptamer sequences to detect ochratoxin a with radiometric fluorescence resonance energy transfer aptasensor [46]. Even same strategy was used for enzyme activity detection [47–49]. Although there are many studies using same methodology, this study is the first turn-off, label free, fluorescence based aptasensor for the detection of ethanolamine.

4. Conclusion

In summary, we have developed a label-free fluorescence aptasensor

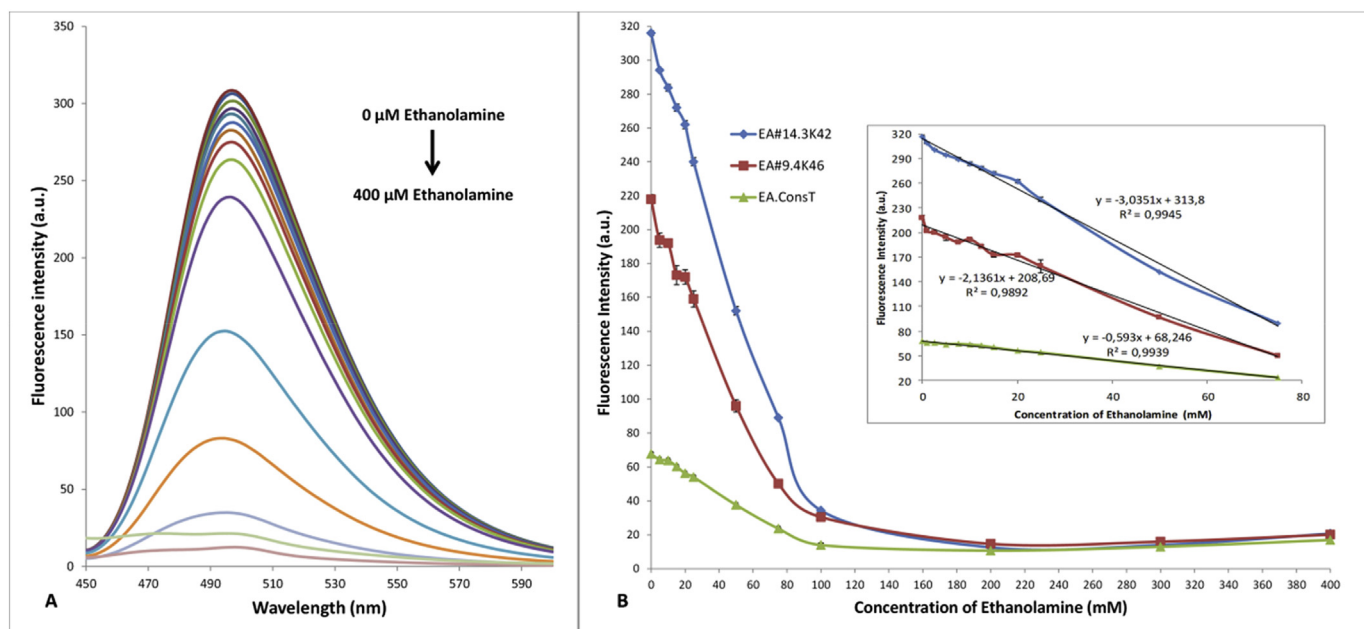


Fig. 4. (A) Fluorescence emission spectra of aptamer-ThT complex in the presence of increasing amounts of ethanolamine (0, 0.5, 1.0, 2.5, 5, 7.5, 10, 12.5, 15, 20, 25, 50, 75, 100, 200, 400 mM). (B) Fluorescence change at 490 nm with the addition of ethanolamine. Inset: linear part of the fluorescence change.

Table 1
Comparison of analytical methods for the detection of ethanolamine.

Sensitivity	Linear Range	Type of Detection	References
5 mM	0.2 mM–1 mM	Electrochemical Biosensor	Skirwar et al. [9]
641 μM	0.5 mM–75 mM	Fluorescence Aptasensor	This work
82 μM	0.3 μM–3 μM	Ion Chromatography	Lehotay et al. [8]
1.2 nM	0.005 μM–5 μM	Gold-nanoparticle Aptasensor	Lee et al. [4]
0,01 nM	0.01 μM–400 μM	Fluorescence Aptasensor	Heilkenbrinker et al. [13]
0.08 pM	0,16 nM–16 nM	Electrochemical Aptasensor	Liang et al. [1]

Table 2
Recovery experiments of ethanolamine in artificially contaminated water samples using the proposed method.

Concentration added (mM)	No FBS added		5% FBS added	
	Concentration found (mM)	Recovery (%)	Concentration found (mM)	Recovery (%)
5	5.5	109.6	5.8	116.2
10	9.9	99.1	10.7	106.6
25	23.9	95.5	27.5	110.0
50	52.3	104.7	55.3	110.6
75	76.3	101.8	81,6	108.8

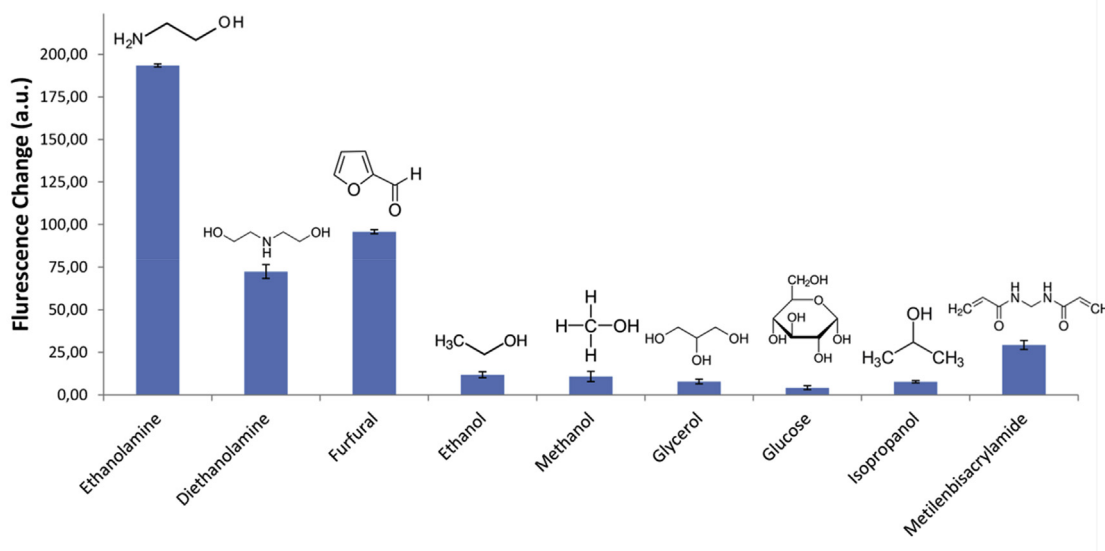


Fig. 5. Specificity of the method for ethanolamine detection.

using ThT-G quadruplex reporter system for sensitive, rapid, simple and wide range detection of ethanolamine. The sensor showed a suitable sensitivity of 140 μM to measure ethanolamine in contaminated water samples in a suitable linear range. Instead of requiring complex experimental techniques, the proposed method is sensitive, selective, low cost compared to chemically labelled fluorescent probes and by the same token works in real samples.

Conflict of interest

The authors report no declarations of interest.

Acknowledgement

The authors would like to gratefully acknowledge the Karamanoglu Mehmetbey University BILTEM and PTTO for their contributions. The authors would like to thank Dr. Ceren Bayrac and Berke Bilgenur Şener for their helpful input.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dyepig.2019.107788>.

References

- Liang G, Man Y, Jin X, Pan L, Liu X. Aptamer-based biosensor for label-free detection of ethanolamine by electrochemical impedance spectroscopy. *Anal Chim Acta* 2016;936:222–8. <https://doi.org/10.1016/j.aca.2016.06.056>.
- Farooqui AA, Rapoport SI, Horrocks LA. Membrane phospholipid alterations in Alzheimer's disease: deficiency of ethanolamine plasmalogens. *Neurochem Res* 1997;22:523–7. <https://doi.org/10.1023/A:1027380331807>.
- Maeba R, Yamazaki Y, Nezu T, Nishimukai M, Okazaki T, Hara H. Serum choline plasmalogen is a novel biomarker for metabolic syndrome and atherosclerosis. *Chem Phys Lipids* 2011;164:S23. <https://doi.org/10.1016/J.CHEMPHYSLIP.2011.05.074>.
- Lee CY, Shiau RJ, Chou HW, Hsieh YZ. Combining aptamer-modified gold nanoparticles with barcode DNA sequence amplification for indirect analysis of ethanolamine. *Sens Actuators B Chem* 2018;254:189–96. <https://doi.org/10.1016/j.snb.2017.07.073>.
- Horrobin DF. The membrane phospholipid hypothesis as a biochemical basis for the neurodevelopmental concept of schizophrenia. *Schizophr Res* 1998;30:193–208. [https://doi.org/10.1016/S0920-9964\(97\)00151-5](https://doi.org/10.1016/S0920-9964(97)00151-5).
- Ogawa S, Hattori K, Sasayama D, Yokota Y, Matsumura R, Matsuo J, et al. Reduced cerebrospinal fluid ethanolamine concentration in major depressive disorder. *Sci Rep* 2015;5:1–8. <https://doi.org/10.1038/srep07796>.
- Cole DEC, Farag S, Dooley KC. Ethanolaminuria: a non-specific laboratory finding in the seriously ill infant. *Clin Biochem* 1988;21:297–300. [https://doi.org/10.1016/S0009-9120\(88\)80084-5](https://doi.org/10.1016/S0009-9120(88)80084-5).
- Lehotay J, Hatrick Š, Motošická A. Trace analysis of ethanolamine in water using ion pair chromatography with on-line preconcentration. *J Liq Chromatogr* 1995;18:1647–54. <https://doi.org/10.1080/10826079508009301>.
- Sikarwar B, Sharma PK, Tripathi BK, Boopathi M, Singh B, Jaiswal YK. Enzyme based electrochemical biosensor for ethanolamine. *Electroanalysis* 2016;28:881–9. <https://doi.org/10.1002/elan.201501046>.
- Gad SC. Ethanolamine. *Encycl. Toxicol. third ed.* Elsevier; 2014. p. 492–5. <https://doi.org/10.1016/B978-0-12-386454-3.00501-7>.
- Mrklas O, Chu A, Lunn S. Determination of ethanolamine, ethylene glycol and triethylene glycol by ion chromatography for laboratory and field biodegradation studies. *J Environ Monit* 2003;5:336–40. <https://doi.org/10.1039/b210572a>.
- Fechner P, Bleher O, Ewald M, Freudenberger K, Furi D, Hilbig U, et al. Size does matter! Label-free detection of small molecule–protein interaction. *Anal Bioanal Chem* 2014;406:4033–51. <https://doi.org/10.1007/s00216-014-7834-4>.
- Heilkenbrinker A, Reinemann C, Stoltenburg R, Walter JG, Jochums A, Stahl F, et al. Identification of the target binding site of ethanolamine-binding aptamers and its exploitation for ethanolamine detection. *Anal Chem* 2015;87:677–85. <https://doi.org/10.1021/ac5034819>.
- Ameen S, Shaheer Akhtar M, Shin H-S. Low temperature grown ZnO nanotubes as smart sensing electrode for the effective detection of ethanolamine chemical. *Mater Lett* 2013;106:254–8. <https://doi.org/10.1016/J.MATLET.2013.05.031>.
- Mairal T, Cengiz Özalp V, Lozano Sánchez P, Mir M, Katakis I, O'Sullivan CK. Aptamers: molecular tools for analytical applications. *Anal Bioanal Chem* 2008;390:989–1007. <https://doi.org/10.1007/s00216-007-1346-4>.
- Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* 1990;249:505–10.
- Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. *Nature* 1990;346:818–22. <https://doi.org/10.1038/346818a0>.
- Robertson DL, Joyce GF. Selection in vitro of an RNA enzyme that specifically cleaves single-stranded DNA. *Nature* 1990;344:467–8. <https://doi.org/10.1038/344467a0>.
- Alsager OA, Kumar S, Zhu B, Travas-Sejdic J, McNatty KP, Hodgkiss JM. Ultrasensitive colorimetric detection of 17-estradiol: the effect of shortening dna aptamer sequences. *Anal Chem* 2015;87:4201–9. <https://doi.org/10.1021/acs.analchem.5b00335>.
- Malhotra S, Pandey AK, Rajput YS, Sharma R. Selection of aptamers for aflatoxin M1 and their characterization. *J Mol Recognit* 2014;27:493–500. <https://doi.org/10.1002/jmr.2370>.
- Bayat P, Nosrati R, Alibolandi M, Rafatpanah H, Abnous K, Khedri M, et al. SELEX methods on the road to protein targeting with nucleic acid aptamers. *Biochimie* 2018;154:132–55. <https://doi.org/10.1016/J.BIOCHI.2018.09.001>.
- Bruno JG, Kiel JL. In vitro selection of DNA aptamers to anthrax spores with electrochemiluminescence detection. *Biosens Bioelectron* 1999;14:457–64.
- Lee YJ, Han SR, Maeng J-S, Cho Y-J, Lee S-W. In vitro selection of Escherichia coli O157:H7-specific RNA aptamer. *Biochem Biophys Res Commun* 2012;417:414–20. <https://doi.org/10.1016/j.bbrc.2011.11.130>.
- Bayrac C, Oktem HA. Evaluation of Staphylococcus aureus DNA aptamer by enzyme-linked aptamer assay and isothermal titration calorimetry. *J Mol Recognit* 2017;30:1–8. <https://doi.org/10.1002/jmr.2583>.
- Bayrac AT, Donmez SI. Selection of DNA aptamers to Streptococcus pneumoniae and fabrication of graphene oxide based fluorescent assay. *Anal Biochem* 2018;556:91–8. <https://doi.org/10.1016/j.ab.2018.06.024>.
- Yunfei Zhang, Yan Chen, Da Han, et al. Aptamers selected by cell-SELEX for application in cancer studies. *Bioanalysis* 2010;2:907–18. <https://doi.org/10.4155/bio.10.46>.
- Bayrac AT, Sefah K, Parekh P, Bayrac C, Gulbakan B, Oktem HA, et al. In vitro selection of DNA aptamers to glioblastoma multiforme. *ACS Chem Neurosci* 2011;2:175–81.
- Sefah K, Shanguan D, Xiong X, O'Donoghue MB, Tan W. Development of DNA aptamers using Cell-SELEX. *Nat Protoc* 2010;5:1169–85. <https://doi.org/10.1038/nprot.2010.66>.
- Jiménez E, Sefah K, López-Colón D, Van Simaey D, Chen HW, Tockman MS, et al. Generation of lung adenocarcinoma DNA aptamers for cancer studies. *PLoS One* 2012;7:e46222. <https://doi.org/10.1371/journal.pone.0046222>.
- Long Y, Qin Z, Duan M, Li S, Wu X, Lin W, et al. Screening and identification of DNA aptamers toward Schistosoma japonicum eggs via SELEX. *Sci Rep* 2016;6:24986. <https://doi.org/10.1038/srep24986>.
- Wang H, Li X, Volk DE, Lokesh GL-R, Elizondo-Riojas M-A, Li L, et al. Morph-X-Select: morphology-based tissue aptamer selection for ovarian cancer biomarker discovery. *Biotechniques* 2016;61:249–59. <https://doi.org/10.2144/000114473>.
- Mann D, Reinemann C, Stoltenburg R, Strehlitz B. In vitro selection of DNA aptamers binding ethanolamine. *Biochem Biophys Res Commun* 2005;338:1928–34.
- Reinemann C, Stoltenburg R, Strehlitz B. Investigations on the specificity of DNA aptamers binding to ethanolamine. *Anal Chem* 2009;81:3973–8. <https://doi.org/10.1021/ac900305y>.
- Chu B, Zhang D, Hwang W, Paukstelis PJ. Crystal structure of a tetrameric DNA fold-back quadruplex. *J Am Chem Soc* 2018;140:16291–8. <https://doi.org/10.1021/jacs.8b10153>.
- Bağda E, Bağda E, Yabaş E. A versatile water-soluble ball-type phthalocyanine as potential antiproliferative drug: the interaction with G-quadruplex formed from tel 21 and cMYC. *JOTCSA* 2017;4:103–23. <https://doi.org/10.18596/jotcsa.288284>.
- Li T, Dong S, Wang E. G-Quadruplex aptamers with peroxidase-like DNzyme functions: which is the best and how does it work? *Chem Asian J* 2009;4:918–22. <https://doi.org/10.1002/asia.200900019>.
- Yeasmin Khusbu F, Zhou X, Chen H, Ma C, Wang K. Thioflavin T as a fluorescence probe for biosensing applications. *TrAC Trends Anal Chem (Reference Ed)* 2018;109:1–18. <https://doi.org/10.1016/j.trac.2018.09.013>.
- Zhu J, Yan Z, Zhou W, Liu C, Wang J, Wang E. Lighting up the thioflavin T by parallel-stranded TG(GA)_n DNA homoduplexes. *ACS Sens* 2018;3:1056–217. <https://doi.org/10.1021/acssens.8b00141>.
- Zhu Y, Li W, Tan S, Chen T. Label-free and simple G-quadruplex-based turn-off fluorescence assay for the detection of kanamycin. *Anal Lett* 2018;51:1718–29. <https://doi.org/10.1080/00032719.2017.1387136>.
- Gogoleva SD, Kalganova EV, Maskevich AA, Lugovskiy AA, Kuzmitsky VA, Goswami M, et al. Neutral derivatives of Thioflavin T do not exhibit viscosity-dependent fluorescence. *J Photochem Photobiol A Chem* 2018;358:76–91. <https://doi.org/10.1016/j.jphtchem.2018.03.003>.
- Wang S, Zhao J, Lu S, Sun J, Yang X. A duplex connection can further illuminate G-quadruplex/crystal violet complex. *Chem Commun* 2019;55:1911–4. <https://doi.org/10.1039/c8cc09940e>.
- Wang Z, Liu J-P. Effects of the central potassium ions on the G-quadruplex and stabilizer binding. *J Mol Graph Model* 2017;72:168–77. <https://doi.org/10.1016/j.jmgm.2017.01.006>.
- Luo D, Mu Y. All-atomic simulations on human telomeric G-quadruplex DNA binding with thioflavin T. *J Phys Chem B* 2015;119:4955–67. <https://doi.org/10.1021/acs.jpcc.5b01107>.
- Bakalova S, Mincheva V, Doycheva A, Groudeva V, Dimkov R. Microbial toxicity of ethanolamines. *Biotechnol Bioelectron Equip* 2008;22:716–20. <https://doi.org/10.1080/13102818.2008.10817540>.
- Wu K, Ma C, Zhao H, He H, Chen H. Label-free G-quadruplex aptamer fluorescence assay for ochratoxin A using a thioflavin T probe. *Toxins* 2018;10(5):198. <https://doi.org/10.3390/toxins10050198>.
- Zeng H, Zhu Y, Ma L, Xia X, Li Y, Ren Y, et al. G-quadruplex specific dye-based ratiometric FRET aptasensor for robust and ultrafast detection of toxin. *Dyes Pigments* 2019;164:35–42. <https://doi.org/10.1016/j.dyepig.2019.01.005>.

- [47] Zhao H, Ma C, Chen M. A novel fluorometric method for inorganic pyrophosphatase detection based on G-quadruplex-thioflavin T. *Mol Cell Probes* 2019;43:29–33. <https://doi.org/10.1016/j.mcp.2018.12.003>.
- [48] Wang T, Que H, Cheng W, Yan X, Ma H, Liu P, et al. Homogeneous fluorescent biosensing method for DNA methyltransferase activity analysis and inhibitor screening based on highly efficient isothermal amplification. *Sens Actuators B Chem* 2019;296:126658. <https://doi.org/10.1016/j.snb.2019.126658>.
- [49] Tang Z, Zhang H, Ma C, Gu P, Zhang G, Wu K, et al. Colorimetric determination of the activity of alkaline phosphatase based on the use of Cu(II)-modulated G-quadruplex-based DNAzymes. *Microchim Acta* 2018;185:109. <https://doi.org/10.1007/s00604-017-2628-y>.