

Progress in the Application of Genetic Engineering in Life Detection

Ziyuan Wang

College of Marine Life, Ocean University of China, Qingdao, Shandong

Abstract. Since December 2019, the outbreak of SARS-CoV-2 has become a major public health challenge, which has a significant impact on the life, health and production of people around the world. Aflatoxin and kanamycin, which can enter the human body through food, and fluoride, which is widely present in the daily environment, are strong carcinogens. Therefore, the detection with high sensitivity, low detection limit and simple operation is needed for those pathogenic bacteria and compounds that endanger human health in life. However, the traditional laboratory operation has some defects, such as the need for expensive instruments, cumbersome operation and a certain demand for professionals. The rapid development of genetic engineering provides an excellent opportunity for the innovation of detection methods. In this paper, the contribution of genetic engineering in detection is briefly reviewed.

Keywords: COVID-19, genetic engineering, aptamer, fluorescent signal, detection method, aflatoxin, kanamycin, fluoride.

1. Introduction

In December 2019, COVID-19 broke out in Wuhan, Hubei Province, China, posing a great threat to human survival and health. On February 11, 2020, it was officially renamed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses, and WHO officially named this viral disease COVID-19; A disease characterized by respiratory distress, fever, cough, fatigue, pneumonia, and muscle pain. With the increasing number of positive cases in China, on January 30, 2020, the World Health Organization declared the virus epidemic as a public health emergency of international concern [1]. Therefore, finding a suitable detection method for COVID-19 has become a top priority for many researchers. Currently, real-time RT-PCR assays are commonly used for the diagnosis of SARS-CoV-2. But unfortunately, in reality, this method is due to sample collection, sample transportation, RNA extraction, EnzymeAnd is easy to cause false positive. Moreover, RT-PCR detection has many limitations due to its heavy workload, the need for skilled operators to detect and collect samples, the need for expensive instruments and special operating sites [2]. Most of these limitations are also common problems in traditional laboratory testing. Therefore, the outbreak of COVID-19 has promoted the innovation of detection methods for all kinds of substances in life.

Genetic engineering is the use of molecular biology technology to modify the DNA sequence in the genome by a variety of methods and to modify the genome by a variety of types of genes. These include knock-outs (DNA sequence deletions), knock-ins (DNA sequence insertions), and substitutions (replacement of a DNA sequence with an exogenous sequence). Short deletions in the genome can be used to remove regulatory elements that knock down gene expression, activate gene expression, or alter protein structure/function by altering the coding sequence [3]. At present, the hottest research on genetic engineering includes the structure and function of aptamers, the linkage of CRISPR technology with non-homologous end joining (NHEJ) repair or homology-directed repair (HDR), locus-specific genetic engineering vectors in mouse and rat zygotes, the selection of viruses and transposons as genetic engineering vectors, and the use of adenovirus for gene targeting. With the rise of the field of synthetic biology, genetic engineering and synthetic biology complement each other and play an indispensable role in many research fields. For example, fluorescent proteins are used in the characterization of reporter models, and Cre-loxP systems and FLP recombinases are used to regulate the expression of foreign genes [4]. Tools for engineering bacteria and fungi (yeast and filamentous fungi) as well as tools that can be used to construct metagenomic libraries will be

constructed, and modular vectors with standardized components and orthogonally designed circuits will be developed [5].

Therefore, it is a good way to design detection methods for pathogenic microorganisms and other substances in life by means of genetic engineering. This is also true of many studies. This article will start with the novel coronavirus that has a significant impact on human production and life in the past three years, and introduce the application of several genetic engineering in the detection of novel coronavirus and the related detection methods of compounds that have a greater impact on human health, including aflatoxin, kanamycin and fluoride.

2. Application of genetic engineering in COVID-19

Severe Acute Respiratory Syndrome (SARS) coronavirus 2 (SARS-CoV-2) has spread rapidly since it was discovered in December 2019, which has a serious impact on people's lives and health around the world [6] and has become a major public health challenge [7]. By mid-2022, the SARS-CoV-2 virus has infected more than 515 million people, of whom 6,255,965 have died; meanwhile, the virus has multiple variants of Beta, Delta and Omicron [8]. At present, a variety of diagnostic methods for SARS-CoV-2 have been proposed, mainly focusing on the detection of SARS-CoV-2 nucleic acid or antibody. Real-time quantitative polymerase chain reaction (RT-qPCR) is used for nucleic acid detection, which has extremely high sensitivity and specificity, but this laboratory-based detection method has the limitations of high cost, long turnaround time, limited number of samples that can be processed, and the need for sophisticated equipment and skilled technicians. The detection of antibodies can only be detected long after the human body is infected with the virus, which greatly affects the timeliness of virus detection and increases the risk of deterioration of the patient's condition. Early diagnosis of COVID-19 is the most effective way to control the spread of the disease and prevent the rise of mortality rate. And now with China's full liberalization of the epidemic, the world seems to have entered a post-COVID era, for the general public, should actively do a good job of personal protection and timely self-detection of the new coronavirus. Therefore, there is still a great need to explore alternative biomarkers for SARS-CoV-2 diagnosis and develop matching assays.

2.1. Application of genetic engineering in detection of novel coronavirus

Since the outbreak of COVID-19, there have been endless tests for COVID-19, but different tests vary in price, timeliness and sensitivity. At present, the commonly used detection method of COVID-19 is based on the detection of virus nucleic acid sequence, using the traditional method of PCR to amplify samples, which involves cumbersome steps, time-consuming, high price, and cannot target the detection of live virus. Obviously, there is a need for some fast, simple, affordable and sensitive methods to detect COVID-19, which will be favored by more people.

2.1.1. DNA network fluorescent sensor

A recent study proposed a "three-layer" Designer DNA Nanostructure design strategy [9], which can quickly and sensitively detect COVID-19, and can also specifically detect the presence of live COVID-19. When there is a live novel coronavirus in the body of the examinee, in addition to detecting the positive results, it is also necessary to carry out an early control of the novel coronavirus in a timely manner. DNA aptamers with fluorescent reporter genes are designed into a network structure, and each aptamer forms partial complementary pairing with a locked DNA containing a quencher to form a double strand, that is, under normal conditions, the fluorescence of the system is quenched and no fluorescent signal can be detected; However, if the sample of the examinee contains live COVID-19 virus, the DNA aptamer network will automatically bend to adapt to the curvature of the live virus through pattern matching and multivalent interaction, and specifically bind to the spike protein on the surface of the virus, resulting in the aptamer being separated from the locked DNA and emitting fluorescence. Therefore, the amount of the novel coronavirus in the body of a positive examinee can be reflected through the strength of a fluorescent signal; and the DNA aptamer network covers the surface of the novel coronavirus, and can also block the combination between the spike

protein of the virus and a host receptor, so that the damage of the novel virus to a human body can be slowed down in a short time, and time can be gained for the effective treatment of a positive patient.

2.1.2. DNA Biped Walker

In recent years, with the development of nanotechnology, DNA nanotechnology has been adopted by many research institutes. It is an artificial molecular device inspired by nature. A DNA bipedal Walker with dumbbell-wheel structure transition has been designed by using DNA nanotechnology [10], which greatly improves the sensitivity of nucleic acid detection through strand displacement amplification (SDA). A DNA triplex nanostructure [11] which can cause mismatching due to pH change is modified on the surface of an electrode, an SDA primer and a template aiming at a novel coronavirus nucleic acid sequence are combined into one to design a hairpin-shaped probe, and as long as a sample to be tested contains a trace amount of novel coronavirus nucleic acid, the hairpin-shaped probe is amplified to amplify a trace amount of novel coronavirus nuclear acid signal, At the same time, the dumbbell-shaped probe sequence was activated to trigger the bipedal Walker based on DNA wheel, which released the electrochemical tracer modified on the triplex after combining with the partial sequence of the triplex, thus reducing the electrochemical response. The amount of viral nucleic acid is reflected by the reduction of electrochemical response per unit time, and small changes in the content of novel coronavirus nucleic acid can be detected. The method can detect even a very small amount of novel coronavirus nucleic acid samples, and has the advantages of high sensitivity, simple operation, strong specificity and high detection efficiency.

2.1.3. SHERLOCK technology

With CRISPR technology winning the Nobel Prize, it has also played a corresponding role in COVID-19 nucleic acid detection in the face of the outbreak of COVID-19. Among them, Professor Zhang Feng's team transformed CRISPR-Cas13a into SHERLOCK technology with sensitivity up to aM, which can detect single molecules [12], and combined with engineering, provided ideas for the manufacture of small devices for self-detection of novel coronavirus for home use [13]. When there is novel coronavirus nucleic acid in the sample of the examinee, it will combine with the complementary sequence of novel coronavirus nucleic acid designed in advance, so as to activate the cleavage activity of CRISPR-Cas13a, cut the connection sequence between the fluorescent group and the quenching group, separate them and emit fluorescence, which can detect whether the examinee is infected with novel coronavirus according to the strength of the fluorescence signal. The method has high sensitivity, simple and rapid operation, and can realize the field detection of novel coronavirus.

2.1.4. DNA Polyhedron

From October to early December 2022, in order to effectively control the spread of COVID-19, daily nucleic acid testing for COVID-19 has become a normal measure in some parts of mainland China. At present, the most commonly used nucleic acid sampling method is to use cotton swabs to sample the mouth and pharynx, and then take them for testing. The saliva in the mouth, especially when dipped in cotton swabs, may contain a variety of pathogenic bacteria and viruses. Then a nucleic acid sample can be taken from the subject to detect whether he has COVID-19 and other bacteria or viruses that may exist in saliva, such as hepatitis virus, HIV virus, *Helicobacter pylori* and so on. A research team has designed a tetrahedral module of DNA for multiplex analysis of miRNA, endonuclease and aptamer-ligand complex; for both miRNA and endonuclease, fluorescence is excited when the target is present, while for aptamer-ligand complex, fluorescence is quenched when the target is present [14]. If the above studies are used for the detection and analysis of multiple diseases after the novel coronavirus sampling, the DNA tetrahedron needs to be modified. A DNA polyhedral module with relative stability can be design according to that numb of diseases to be detected, a corresponding number of hairpin loop are modified on the edge length of the polyhedron, wherein the stem region of the hairpin is designed at the spatial position of an associated fluorophore/quencher unit, the loop region comprises recognition sequences of a plurality of mRNAs, and when corresponding targets exist, Resulting in the opening of the respective self-clamping

structure and the deformation of the scaffold, and the fluorophore is separated from the quencher and emits fluorescence. However, for those bacteria and viruses that have more significant secretions or structural substances than mRNA to prove their existence, it is obvious that the analysis using aptamers will have higher accuracy and sensitivity. Because the human eye is more sensitive to light than to darkness, the excited fluorescence is better in the presence of the target than the quenched fluorescence in the study. Aptamers with ribozyme properties can be designed to trigger their self-cleavage function when the corresponding ligands exist and bind to them, resulting in the flexibility of the corresponding edges in the DNA polyhedron, the separation of fluorophores and quenchers, and the emission of fluorescence. By detecting the color and intensity of the fluorescence, the virus infected by the examinee and the infection degree can be judged, and the detection can be carried out simply and quickly to gain time for the timely treatment of the examinee.

2.2. Application of genetic engineering in detecting the effect of COVID-19 vaccine injection

Since the outbreak of COVID-19, researchers have intensified their research on vaccines and various types of COVID-19 vaccine have emerged. In the face of many types of vaccines, an important factor that people need to consider when choosing is the effectiveness of vaccines. So how to detect the antibody level in the vaccinated population has become an urgent problem to be solved. At present, the commonly used antibody detection method is lateral flow immunochromatography, but it can only be used for qualitative detection, which hinders their use in therapeutic monitoring and has certain limitations, so accurate quantitative detection is needed for therapeutic monitoring.

2.2.1. Antibody Responsive DNA Circuit

Recent studies have coupled DNA nanotechnology and electrochemical sensors to form an antibody-responsive DNA circuit [15]. When there is antibody in the blood sample of the vaccine recipient, it will bind to the pre-set COVID-19 antigen, induce the bivalent binding of two antigen-modified DNA to form a dimer, and then bind to and invade the toehold part of the pre-hybridized double strand, releasing the redox reporter modified single strand. This strand is then hybridized with a capture strand attached to an electrode to produce an electrochemical signal. The antibody level in that recipient can be quantitatively judged according to the strength of the detected electrochemical signal.

2.2.2. Molecular Timer

Francesco's team proposed an enzyme-based strategy to control the initiation of DNA strand displacement reactions (SDRs) in real time, that is, to hybridize and bind the blocking strand to the toehold region of the target double strand, to degrade the blocking strand after adding specific enzymes, and to expose the toehold region to bind to the input strand, thus initiating the SDR reaction. The time for the enzyme to degrade the blocked chain is the time for the delay to be achieved [16]. This principle can be used to detect the antibody level of novel coronavirus with slight modification. Specific aptamers are designed for COVID-19 antibodies (COVID-19 antibodies as ligands, specific aptamer domains are designed), and the aptamers are designed as input chains with fluorescent labels on the output chains. If there is an antibody in the blood sample of the recipient, the aptamer will bind to the antibody (ligand) to form an aptamer. At this time, because it takes a certain time for the specific enzyme to degrade the blocking chain, almost all aptamers will bind to the antibody instead of replacing the output chain as an input chain, so no fluorescence can be observed. If there is no antibody in the blood sample of the recipient, the aptamer will act as an input strand to replace the output strand, thus generating a fluorescent signal. However, due to the existence of blocking chains, it takes some time for specific enzymes to degrade the blocking chains, and the generation of fluorescence signals has a certain delay. If there is no molecular timer, when the antibody exists, a part of the aptamer will combine with the antibody to form an aptamer, and at the same time, a part of the aptamer will be used as an input chain to replace the output chain, which will immediately produce a fluorescent signal of a certain intensity; when there is no antibody, it will also immediately produce a fluorescent signal, which is not conducive to judging whether there is a novel coronavirus antibody. The existence of molecular timer greatly avoids this misjudgment. Moreover, when the

antibody is present, the antibody level in the recipient can be quantitatively predicted based on the amount of the specific enzyme involved in the reaction. (The amount of enzyme involved in the specificity of the reaction is inversely proportional to the level of antibody. That is, the less the number of specific enzymes involved in the reaction, the more antibodies in the recipient; and vice versa).

3. Application of Genetic Engineering in the Detection of Other Substances in Life

3.1. Application of Genetic Engineering in Detection of Aflatoxin

Food contamination is often referred to as rotten or spoiled food because of the presence of certain harmful microorganisms, including bacteria or parasites, or because of oxidative stress or excessive toxic chemicals leading to spoilage and causing foodborne illness, which severely affects humans and has a significant global public health concern, especially in developing countries [17]. Aflatoxin is the most harmful to human beings in food-borne mycotoxins, which can enter the human body through milk, oil crops and so on. Due to the potential for degradation of aflatoxins and the interaction of the resulting toxic compounds with food components, chronic disease or severe cancer may result, increasing morbidity and mortality rate [18]. Therefore, it is necessary to detect aflatoxin in food. At present, the commonly used detection methods for aflatoxin include high performance liquid chromatography (HPLC), Kjeldahl apparatus, thin layer chromatography (TLC), high performance liquid chromatography-mass spectrometry (HPLC-MS), liquid chromatography-mass spectrometry (LC-MS), Enzyme-linked immunosorbent assay (ELISA) and gas chromatography-mass spectrometry (GC-MS), but these methods have some shortcomings, such as complex sample pretreatment, high detection cost and requiring professional operation. Therefore, the method which is affordable, simple and can detect aflatoxin quickly and accurately will be welcomed by more people.

With the development of genetic engineering, new detection technologies for aflatoxins can make up for the shortcomings of traditional detection technologies, especially the advent of Ramanomics and aptamers, which makes aptamer sensors based on surface-enhanced Raman spectroscopy (SERS) highly sensitive and selective in the detection of aflatoxins. Aptamer sensors with the highest Raman signal were constructed by aptamer recognition of sH-cDNA modified Fe₃O₄ @ AuNFs and sH-Apt modified Au-4MBA @ AgNSs. When there is no aflatoxin in the food to be detected, the highest Raman signal can be obtained due to the multiple SERS effect; When aflatoxin AFB₁ is present in the food to be detected, the aptamer preferentially binds to AFB₁, triggering Fe₃O₄ @ AuNFs to release the reporter probe, resulting in a linear decrease in SERS intensity with the increase of AFB₁ concentration after magnetic separation [19]. According to the intensity of SERS, the presence and concentration of aflatoxin can be judged, so as to realize the simple, rapid and quantitative detection of aflatoxin.

3.2. Application of genetic engineering in the detection of kanamycin

Kanamycin is a basic aminoglycoside antibiotic produced by Kanamycin, which has the ability to block protein synthesis and can be used to treat severe Gram-positive and Gram-negative bacterial infections [20]. Kanamycin can also target cellular RNA to inhibit bacterial and viral replication, and its hydroxyl and amino groups can be hydrogen bonded to specific lipid components of bacterial membranes to disrupt bacterial biofilms [21]. However, the abuse of kanamycin in humans will eventually endanger themselves. For example, kanamycin can remain in animal foods, enter the human body through the food chain and food web, and be toxic to the human body. Recently, kanamycin residues have become a serious public health problem worldwide [20].

At present, the main detection methods for kanamycin residues include high performance liquid chromatography (HPLC), high performance liquid chromatography tandem mass spectrometry (LC-MS), surface plasmon resonance (SPR), enzyme-linked immunosorbent assay (ELISA) and so on.

Although these traditional laboratory methods can achieve the preliminary detection of kanamycin residues, its sensitivity, operational complexity and detection efficiency need to be studied. Therefore, there is an urgent need for methods with high sensitivity, simple operation and rapid detection in the field.

Some studies have used genetic engineering to immobilize kanamycin on the surface of acetaldehyde-based magnetic beads by carbodiimide crosslinking agent to construct magnetic bead complexes. In the absence of kanamycin, the aptamer specifically binds to kanamycin immobilized on magnetic beads with high affinity. When a mixture of TDT, dNTPs, and biotin-dUTP was added, TDT catalyzed the sequential extension of the aptamer 3'-OH-terminal deoxyribonucleoside without any template to generate a long single-stranded DNA (ssDNA) product embedded in the biotin site. With the introduction of streptavidin-labeled horseradish peroxidase, the HRP molecule is incorporated into the single-stranded DNA strand via biotin-SA binding. Finally, HRP catalyzes the hydrogen peroxide-mediated oxidation of TMB and forms a blue chromogenic product for colorimetric analysis. However, when kanamycin is present in the sample to be detected, the aptamer will preferentially bind to the free kanamycin molecule due to its small steric hindrance, resulting in the aptamer being almost consumed, which is then removed by magnetic separation. This means that no 3'-OH terminus is left for subsequent amplification, thus resulting in no effective signal. As the concentration of free target kanamycin decreased, the number of aptamers bound to kanamycin immobilized on magnetic beads increased, resulting in an increase in signal. Therefore, the content of kanamycin in the sample to be tested can be quantitatively detected by the presence and depth of the blue signal, that is, there is a negative correlation between them [20].

For the color index of quantitative detection of kanamycin, in addition to the chromogenic products generated by related reactions, the method of fluorescent markers can also be applied. The fluorescence of gold nanoparticles (AuNP) combined with aptamers has been studied to achieve a simple and sensitive detection of kanamycin. The researchers used AuNP as a DNA nanocarrier as well as a highly efficient fluorescence quencher. When there is no kanamycin in the sample to be detected, the dye labeled aptamer can adsorb to the surface of AuNPs, resulting in quenching of the fluorescence signal. When kanamycin is present, the specific binding of the dye-labeled aptamer to kanamycin induces the formation of a rigid structure, resulting in the release of the dye-labeled aptamer from the surface of AuNPs, thereby restoring the fluorescence intensity [22]. Therefore, the content of kanamycin can be quantitatively detected by the intensity of fluorescence, and the method has the advantages of high speed, high sensitivity and low detection limit in the aspect of detecting kanamycin.

3.3. Application of genetic engineering in fluoride detection

Fluoride ions, as the smallest and most electronegative anions, are among the most difficult targets to be targeted by the corresponding aptamers because they provide only a small number of interaction sites and their electronegativity produces strong electrostatic repulsion with nucleic acids [23]. Fluoride is widely distributed in the environment, occurring in air, soil, rocks, and water. Fluoride is used industrially in the manufacture of fluorochemicals, ceramics, pesticides, aerosol propellants, refrigerants, glassware, and Teflon cookware. Although fluoride has little benefit in reducing dental caries, it is closely related to cognitive impairment, hypothyroidism, dental and skeletal fluorosis, enzyme and electrolyte disorders, and uterine cancer [24]. Therefore, there is a need for a suitable detection method for fluoride with good biocompatibility, high selectivity and non-toxicity.

Ryckelynck's team conducted high-throughput screening through droplet microfluidics and obtained an optimal connection element to construct FluorMango, that is, a biosensor that can be directly used to detect fluoride by directly connecting the aptamer domain of fluoride ion riboswitch to the fluorescent aptamer of RNA [23]. When fluoride is present, it excites fluorescence. Within a certain range, there is a linear relationship between the fluorescence intensity and the fluoride concentration, so that the fluoride concentration can be quantitatively detected.

4. Summary and outlook

To a certain extent, there are similarities in the detection methods reviewed above for pathogenic bacteria and compounds that endanger human health in life. By using the knowledge of molecular biology and synthetic biology, the three-dimensional structure and sequence of aptamers and related nucleic acid sequences can be modified to expand the scope of application of genetic engineering in detection. Achieve simpler, faster, and more sensitive detection.

Under the trend that the integration of disciplines has become the mainstream, interdisciplinary research methods will be favored by more and more people. Related research in the field of genetic engineering can be combined with mathematics, physics, computer, big data and other fields to optimize biological methods with stronger mathematical logic. Recently, the optical properties of liquid crystals have been used to detect target molecules. By developing a novel LC biosensor platform based on the self-assembly of oligonucleotide chains in the P-conformation immobilized on a treated glass substrate for the precise determination of OTA. The rigid and asymmetric P-shaped DNA structure greatly disrupts the orientation of the LC, making it a random arrangement, and then the orientation change of the liquid crystal leads to a new signal readout, realizing the optical appearance of the polarized image from light to dark transition [25].

In addition to the above, there are many detection methods for COVID-19, and their common purpose is to make the detection more sensitive, cheaper, simpler and faster, and easier to use. The above detection methods for COVID-19 can also be used in the pre-diagnosis of other infectious diseases and cancer. I believe that in the near future, with the further development of science and technology, human beings will be calmer in dealing with public health diseases and have more confidence to overcome terrible diseases. Of course, for all mankind, we should work together to protect the earth's ecological environment, strengthen exercise to enhance their immunity, reduce the occurrence of infectious diseases and the risk of fatal diseases.

References

- [1] Fernandes, Q., Inchakalody, V.P., Merhi, M., Mestiri, S., Taib, N., Moustafa Abo El-Ella, D., Bedhiafi, T., Raza, A., Al-Zaidan, L., Mohsen, M.O., Yousuf Al-Nesf, M.A., Hssain, A.A., Yassine, H.M., Bachmann, M.F., Uddin, S. and Dermime, S. (2022). Emerging COVID-19 variants and their impact on SARS-CoV-2 diagnosis, therapeutics and vaccines. *Annals of Medicine*, [online] 54 (1), pp.524 – 540. doi:10.1080/07853890.2022.2031274.
- [2] Benefits and limitations of serological assays in COVID-19 infection. (2020). *Indian Journal of Tuberculosis*. [online] doi: 10.1016/j.ijtb.2020.07.034.
- [3] Lanigan, T.M., Kopera, H.C. and Saunders, T.L. (2020). Principles of Genetic Engineering. *Genes*, [online] 11 (3), p.291. doi:10.3390/genes11030291.
- [4] Friedland, A.E., Lu, T.K., Wang, X., Shi, D., Church, G. and Collins, J.J. (2009). Synthetic Gene Networks That Count. *Science*, 324 (5931), pp.1199 – 1202. doi:10.1126/science.1172005.
- [5] Nora, L.C., Westmann, C.A., Martins-Santana, L., Alves, L. de F., Monteiro, L.M.O., Guazzaroni, M.-E. and Silva-Rocha, R. (2018). The art of vector engineering: towards the construction of next-generation genetic tools. *Microbial Biotechnology*, 12 (1), pp.125 – 147. doi:10.1111/1751 - 7915.13318.
- [6] Liang, Q., Huang, Y., Wang, M., Kuang, D., Yang, J., Yi, Y., Shi, H., Li, J., Yang, J. and Li, G. (2023). An electrochemical biosensor for SARS-CoV-2 detection via its papain-like cysteine protease and the protease inhibitor screening. *Chemical Engineering Journal (Lausanne, Switzerland: 1996)*, [online] 452, p.139646. doi: 10.1016/j.cej.2022.139646.
- [7] A rapid diagnosis of SARS-CoV-2 using DNA hydrogel formation on microfluidic pores. (2021). *Biosensors and Bioelectronics*, [online] 177, p.113005. doi: 10.1016/j.bios.2021.113005.
- [8] Ji, D., Guo, M., Wu, Y., Liu, W., Luo, S., Wang, X., Kang, H., Chen, Y., Dai, C., Kong, D., Ma, H., Liu, Y. and Wei, D. (2022). Electrochemical Detection of a Few Copies of Unamplified SARS-CoV-2 Nucleic Acids by a Self-Actuated Molecular System. *Journal of the American Chemical Society*, [online] 144(30), pp.13526–13537. doi:10.1021/jacs.2c02884.

- [9] Chauhan, N., Xiong, Y., Ren, S., Dwivedy, A., Magazine, N., Zhou, L., Jin, X., Zhang, T., Cunningham, B.T., Yao, S., Huang, W. and Wang, X. (2022). Net-Shaped DNA Nanostructures Designed for Rapid/Sensitive Detection and Potential Inhibition of the SARS-CoV-2 Virus. *Journal of the American Chemical Society*, [online] p. jacs.2c04835. doi:10.1021/jacs.2c04835.
- [10] Miao, P., Chai, H. and Tang, Y. (2022). DNA Hairpins and Dumbbell-Wheel Transitions Amplified Walking Nanomachine for Ultrasensitive Nucleic Acid Detection. *ACS nano*, [online] 16 (3), pp.4726 – 4733. doi:10.1021/acsnano.1c11582.
- [11] Thompson, I.A.P., Zheng, L., Eisenstein, M. and Soh, H.T. (2020). Rational design of aptamer switches with programmable pH response. *Nature Communications*, 11(1). doi:10.1038/s41467-020-16808-2.
- [12] Gootenberg, J.S., Abudayyeh, O.O., Lee, J.W., Essletzbichler, P., Dy, A.J., Joung, J., Verdine, V., Donghia, N., Daringer, N.M., Freije, C.A., Myhrvold, C., Bhattacharyya, R.P., Livny, J., Regev, A., Koonin, E.V., Hung, D.T., Sabeti, P.C., Collins, J.J. and Zhang, F. (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science*, 356(6336), pp.438 – 442. doi:10.1126/science.aam9321.
- [13] Puig, H. de, Lee, R.A., Najjar, D., Tan, X., Soekensen, L.R., Angenent-Mari, N.M., Donghia, N.M., Weckman, N.E., Ory, A., Ng, C.F., Nguyen, P.Q., Mao, A.S., Ferrante, T.C., Lansberry, G., Sallum, H., Niemi, J. and Collins, J.J. (2021). Minimally instrumented SHERLOCK (miSHERLOCK) for CRISPR-based point-of-care diagnosis of SARS-CoV-2 and emerging variants. *Science Advances*, [online] 7 (32), p. eabh2944. doi: 10.1126/sciadv. abh2944.
- [14] Zhou, Z., Sohn, Y.S., Nechushtai, R. and Willner, I. (2020). DNA Tetrahedra Modules as Versatile Optical Sensing Platforms for Multiplexed Analysis of miRNAs, Endonucleases, and Aptamer-Ligand Complexes. *ACS nano*, [online] 14 (7), pp.9021 – 9031. doi: 10.1021/acsnano.0c04031.
- [15] Bracaglia, S., Ranallo, S., Plaxco, K.W. and Ricci, F. (2021). Programmable, Multiplexed DNA Circuits Supporting Clinically Relevant, Electrochemical Antibody Detection. *ACS Sensors*, 6 (6), pp.2442 – 2448. doi:10.1021/acssensors.1c00790.
- [16] Bucci, J., Irmisch, P., Del Grosso, E., Seidel, R. and Ricci, F. (2022). Orthogonal Enzyme-Driven Timers for DNA Strand Displacement Reactions. *Journal of the American Chemical Society*, [online] 144 (43), pp.19791 – 19798. doi: 10.1021/jacs.2c06599.
- [17] Parihar, A., Choudhary, N.K., Sharma, P. and Khan, R. (2023). MXene-based aptasensor for the detection of aflatoxin in food and agricultural products. *Environmental Pollution (Barking, Essex: 1987)*, [online] 316(Pt 1), p.120695. doi: 10.1016/j.envpol.2022.120695.
- [18] Yin, S., Niu, L. and Liu, Y. (2022). Recent Progress on Techniques in the Detection of Aflatoxin B1 in Edible Oil: A Mini Review. *Molecules*, 27(19), p.6141. doi:10.3390/molecules27196141.
- [19] He, H., Sun, D.-W., Pu, H. and Huang, L. (2020). Bridging Fe₃O₄@Au nanoflowers and Au@Ag nanospheres with aptamer for ultrasensitive SERS detection of aflatoxin B1. *Food Chemistry*, 324, p.126832. doi: 10.1016/j.foodchem.2020.126832.
- [20] Zhao, T., Chen, Q., Wen, Y., Bian, X., Tao, Q., Liu, G. and Yan, J. (2022). A competitive colorimetric aptasensor for simple and sensitive detection of kanamycin based on terminal deoxynucleotidyl transferase-mediated signal amplification strategy. *Food Chemistry*, [online] 377, p.132072. doi: 10.1016/j.foodchem.2022.132072.
- [21] John, T., Thomas, T., Abel, B., Wood, B.R., Chalmers, D.K. and Martin, L.L. (2017). How kanamycin A interacts with bacterial and mammalian mimetic membranes. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1859 (11), pp.2242 – 2252. doi: 10.1016/j.bbamem.2017.08.016.
- [22] Chen, J., Li, Z., Ge, J., Yang, R., Zhang, L., Qu, L.-B., Wang, H.-Q. and Zhang, L. (2015). An aptamer-based signal-on bio-assay for sensitive and selective detection of Kanamycin A by using gold nanoparticles. *Talanta*, [online] 139, pp.226 – 232. doi: 10.1016/j.talanta.2015.02.036.
- [23] Husser, C., Vuilleumier, S. and Ryckelynck, M. (2022). FluorMango, an RNA-Based Fluorogenic Biosensor for the Direct and Specific Detection of Fluoride. *Small (Weinheim an Der Bergstrasse, Germany)*, [online] p.e2205232. doi:10.1002/smll.202205232.
- [24] Peckham, S. and Awofeso, N. (2014). Water Fluoridation: A Critical Review of the Physiological Effects of Ingested Fluoride as a Public Health Intervention. *The Scientific World Journal*, 2014, pp.1 – 10. doi:10.1155/2014/293019.

- [25] Verdian, A., Khoshbin, Z. and Chen, C.-H. (2022). Development of a novel liquid crystal Aptasensing platform using P-shape molecular switch. *Biosensors & Bioelectronics*, [online] 199, p.113882. doi: 10.1016/j.bios.2021.113882.