Review Paper:

Current and Future Perspectives on the Development and Design of Aptamers

Tandasi Vandana* and Srivastava Sudha
Department of Biotechnology, Jaypee Institute of Information Technology, Noida, Uttar Pradesh, INDIA
*tandasivandana@gmail.com

Abstract

Aptamers are oligonucleotides that display unique characteristics of identifying and binding to certain biological targets with utmost precision. The process of generating aptamers by conventional techniques has been time-consuming, expensive and labour-intensive. To counter these limitations, the emergence of advanced computational and Artificial intelligence (AI) technologies have opened up opportunities for the remarkable growth and advancement of aptamer applications in drug delivery, diagnostics and therapeutics. AI has revolutionised the biomedical field paving a path for development of specific and high affinity aptamers to enhance efficiency, to reduce time, costs and manpower.

In this review, we explore several ongoing studies, various aptamer database developed and patents filed that deepen our understanding of the role of aptamers in biomedical science and several upcoming developments utilizing AI tools and computational algorithms. We also present a comprehensive overview of computational AI tools for aptamer selection, aptamer design, aptamer modelling, aptamer protein binding prediction and the design of aptasensors.

Keywords: Aptamer, Artificial Intelligence, Computational Tools.

Introduction

Aptamers are single-stranded nucleic acids which may be DNA and RNA of short stretches usually 30-60 nucleotide in length. They can fold into a various secondary structure and create specific three-dimensional configuration upon binding. These molecules have the capability of recognizing and binding to target molecules and have a large number of applications: drug delivery, therapeutics and diagnostics. Aptamers can be designed to bind a vast range of targets, from small ions to large proteins and even whole cells or bacteria. They have the capability to differentiate subtle variations between closely related molecules, often differentiating isomers or even enantiomers.

Aptamers have striking similarities to antibodies, in terms of exceptional specificity and affinity for their target molecules. However, aptamers exhibit greater advantages over them for use in targeted drug delivery, the treatment of specific diseases and in diagnostics- particularly biosensing. These short oligonucleotides have greater stability over a wide temperature range and elicit minimal immune responses *in vivo*, making them less immunogenic than antibodies[22,76]. The simpler molecular structures, owing to their nucleotide nature, make them suitable to be synthetically manufactured and easily modified, providing a scalable and adaptable alternative to traditional antibodies.

The extensive applications of aptamers in diagnostics and therapeutics have seen a surge in recent research advancements. Due to improved research and development of aptamers, there have been filed a lot of patents that involve diagnostics, therapeutics and research methodologies to develop aptamers. Numerous patents are based on aptamer sequences and their application in binding to specific targets. A large number patents have been granted on SELEX, the method used for generating aptamers. The growing amounts of biological and chemical datasets, derived from high-throughput experimental studies and screening in many areas like proteomics and genomics, have given rise to many of the aptamer databases.

The database is a useful tool for academic and industrial researchers who design and investigate multiple and diverse aptamers. These databases serve as a repository of sequences that might be useful for diagnosis or treatment. These databases may be openly accessible and updated periodically to maintain the most recent updates in the field.[50] The aptamers are usually developed via an *in vitro* procedure called Systematic Evolution of Ligands by EXponential enrichment (SELEX). The process of developing aptamers is repetitive, labor-intensive and time-consuming which makes the SELEX procedures less preferred in recent times.

The huge amount of generated data has also been incorporated with AI into several phases of research from identifying targets to designing clinical trials. Large volumes of biological data can be efficiently processed, structured and stored by AI-based algorithms, allowing for its rapid analysis, which is a basic requirement for creating an intelligent computer system with systematic functionality to comprehend the data[72,82]. AI has emerged as a crucial instrument in recent years for faster customizing therapies and drug discovery, indicating an evolution towards more effective, data-driven pharmaceutical research, completely transforming the biomedical industry and leading to the development of accurate, highly-affinity aptamers while cutting expenses, time and labour.

The AI-based novel computational methods which include machine learning, deep learning (ML/DL) algorithms, have

enabled the developers to identify aptamer candidates with high affinity and specificity to the target molecules in drug development. It has been proved that a few ML DL techniques perform better than a variety of traditional screening and binding affinity prediction techniques including molecular docking and virtual screening. This potential has recently been uncovered after the research on the intersection of engineering biology and ML/DL tools in the design of prediction of new molecular structures, best possible experimental design, automatic microscopy data analysis and biomolecular implementations of ANNs (Artificial Neural Networks)[26]. MEMERIS, AptaMotif, MPBind and AptaTrace are just a few of the several motif-determining tools that have been developed to find sequence and/or structural trends within a group of potential aptamers[10,72].

In this review, we have discussed the importance and progress of aptamers in biomedical studies. Numerous studies and extensive application of aptamers have enabled designing various aptamer databases. A large number of patents have been already granted on aptamers that were developed against the target protein and the process of aptamer generation. These developments help us better to understand the function of aptamers and their significance in biomedical research. We have also discussed about various aptamer-based AI based tools and computational methods are useful for *in silico* development, identification and selection of the aptamers.

Progress of aptamers in the biomedical science

The ability of aptamers to specifically recognize and bind to target molecules has led to their widespread use in a variety of industries. As a result, they are increasingly being used for clinical diagnostics and therapy, especially in fields like cancer diagnosis, pathogen detection and biosensors. Aptamer science has fascinated attention in the areas of diagnostics, antiviral therapy, cancer detection, regenerative medicine[56].

Current research on aptamers: The research and development (R & D) landscape for aptamers has been dynamic, with numerous ground-breaking advancements spanning multiple disciplines. The aptamer field is gradually collaborating with nanotechnology and materials science to develop multifunctional aptamer-based systems. An overview of some of the recent trends and significant developments in aptamer research has been discussed here.

As promising therapeutic tool: Due to their small size, aptamers have higher permeability and lower immunogenicity than antibodies which result in superior biocompatibility. The chemical integrity and bioavailability of the aptamers when applied in physiological conditions, are beneficial for the therapeutic purpose. Chemical modification of aptamers increases their therapeutic efficacy. As a result, aptamer-based therapies have developed rapidly in recent years. In one of the studies by Affinito et al[1], two RNA aptamers, 40L and A40s could bind to the cell surface of human GSCs. These two aptamers inhibit GSCs' ability to proliferate, remain stem-like and migrate. The *in vitro* investigations demonstrated their ability to pass the blood-brain barrier (BBB) and their stability in serum. These findings imply that the aptamers 40L and A40s are novel, promising therapeutic options for GBM.

In another study, the cell surface receptor protein known as low-density lipoprotein receptor (LDL-R) is expressed in a number of solid tumors, such as those of the liver, brain, colon, lung and colon. LDL-R has the ability to transport lysosome-sensitive anticancer drugs, such as nucleic acid-based therapeutic molecules. Using 10 rounds of the SELEX, Wang et al[79] generated a novel DNA oligonucleotide aptamer, RVN-L7, which is specific to LDL-R. High affinity and specificity were demonstrated by this aptamer (Kd = 19.6 nM) having a binding affinity of about 200 nM. The development of targeted cancer therapies could be beneficial with the use of such therapeutic molecules.

Aptamers in combination therapy: The therapeutic potential of aptamers is boosted by their versatility, which enables them to be integrated into combination therapies. These treatments may inhibit tumor growth and metastasis, may lower systemic toxicity and may result in new methods of cancer treatment such as aptamer-mediated immune cell facilitating immunotherapy, aptamer-conjugated drug delivery systems and aptamer radiation synergy. In one study, the radio-sensitization outcome due to 4MeV electron radiation on the cancer cells was tested using gold nanoparticles (GNP) conjugated with the AS1411 aptamer. The combination of data from clonogenic tests and Au cell uptake showed that the aptamer had enhanced radiation-induced cell death via Au absorption. Because of this, cancer cells are more prone to 4 MeV electron beams when AS1411/GNPs are used instead of GNP alone[58,60].

For Cytokine modulation: The aptamers may be able to regulate cytokine levels during cancer treatments in an effective manner as seen in case of Axin-1 siRNAs that are complexed with the 4-1BB-binding aptamer to target CD25. The 4-1BB receptor is expressed by activated CD8+ T lymphocytes. The combination of 4-1BB aptamer and CD25 siRNA, efficiently decreased protein levels and CD25 mRNA in activated CD8+ T cells *in vitro*. By encouraging the growth of long-lasting memory CD8+T cells, treatment improved the antitumor response in mouse models, with localized radiation therapy and cellular vaccination. Aptamers can transport cytokines to the site of tumor, improving immune cell activation and attachment in the tumor microenvironment without any toxicity.[67]

CAR (Chimeric Antigen Receptor)-aptamer: Aptamers have become valuable tools for identifying cancer-linked markers for targeted therapy. T-cell treatments can be

performed due to high specificity of aptamers. For potential clinical-scale cell therapy applications, a novel CAR-aptamer has been identified by Zhou et al[88] that provides a very effective means for enriching and retargeting CAR-T cells. Using a protein-SELEX method, they discovered a number of DNA aptamers (also known as CAR-aptamers or CAR-ap) that accurately bind with the extracellular CD19 CAR domain and display remarkable binding ability independent of the CAR-T or CAR-NK cell preparation techniques.

As drug delivery vehicles targeting cancer cells: Aptamers are being explored as vehicles for drug delivery that targets cancer cells, often in combination with nanoparticles or other drug carriers. Liu et al[55] created a targeted nanovaccine by engrafting functional DNA - CpG oligonucleotide that is an agonist designed for toll-like receptor 9 and an aptamer that targets the intercellular adhesion molecule (ICAM)-3 unique to dendritic cells (DCs) into cell membrane vesicles (CMVs) that were isolated from tumor cells. These CMVs with altered DNA could specifically target DCs and promote their maturation even further. These nanovaccines may effectively halt the growth of tumors by inducing strong antitumor immune responses.

By eradicating most of the tumors, the combination of CMV-based nanovaccines and immune checkpoint inhibition may boost treatment responses and create long-term immunological memory for prevention of tumor recurrence. This study provides a generic platform of DC-targeted cancer immunotherapy by only building functional DNA on the membrane of CMVs extracted from tumor cells.

Incorporating aptamers into wearable devices for continuous monitoring of physiological parameters: Newer Therapeutic Drug Monitoring (TDM) systems that are precise, non-invasive and can display quick turnaround times, are now growing rapidly. For instance, an electrochemical aptamer biosensing patch (μNEAB-patch) that uses microneedles to probe the interstitial fluid (ISF) minimally invasively was developed by Lin and coworkers[54]. They introduced the μNEAB-patch, which offers continuous and real-time measurements of the pharmacokinetics of circulating pharmaceutical compounds. The NEAB-patch is made using a low-cost fabrication method that converts a clinical-grade, shorter needle into a superior quality substrate made of gold nanoparticles for aptamer immobilization and effective electrochemical signal access. It was developed to target the *in vivo* detection of a wide variety of ISF analyte molecules and the potential clinical utility of the device for prompt prediction of the drug exposure.

For super-resolution imaging of proteins: An essential condition for obtaining a high-quality single-molecule localization microscopy image is the adequate fluorophore labelling that does not harm biological targets. This can be easily achieved by using labelled aptamers as compared to antibodies that have large size causing steric hindrances and may cause a linkage error of about 10 to 20 nm. In one such study, Yan et al[83] labeled the membrane EGFR on living cells using a previously described RNA aptamer to enable high-quality dSTORM imaging of EGFR distribution and clustering. In this study, aptamers were shown to be beneficial for adequate fluorophore labeling and highly specific identification, which explained EGFR's more precise and intricate spatial organization.

Aptamers appear to be interesting tools for super-resolution imaging of membrane proteins, as the aptamer recognition method demonstrated the subtle changes in clustering between active and resting EGFR on live cell membranes that have not yet been discovered by antibody labeling. The current R and D landscape for aptamers is vast and multidisciplinary. With their unique properties of specificity, versatility and modifiability, aptamers are at the forefront of numerous innovations in diagnostics, therapeutics, environmental monitoring and beyond. The coming years promise even more exciting developments as the confluence of technologies further expands the horizons for aptamer applications.

Aptamer based patents: Since the discovery of aptamers, a lot of studies have been performed across fields like diagnostics, therapeutics, biosensing and targeted drug delivery as a result of which, numerous patents have been filed. Patents in the category of diagnostic and biosensing cover aptamers that are used as biosensors, molecular probes, or diagnostic tools. For instance, there are patents relating to aptamers that bind specific biomarkers, enabling the detection of diseases like Alzheimer's or cardiovascular disorders.

Drug delivery and targeting patents typically cover aptamers that are conjugated to drugs, nanoparticles, or other therapeutic agents to enable targeted delivery. The aptamer acts as a homing mechanism, guiding the drug to specific cells or tissues. Methodology patent cover improvements or modifications to the SELEX process and other techniques for aptamer identification and optimization. For example, patents might describe new methods for increasing the efficiency of aptamer selection or for introducing modifications to enhance aptamer stability.

The initial patent for the SELEX method was granted to inventors from the University of Colorado and Harvard Medical School. These patents, held by companies like NeXagen and later Gilead Sciences, cover the basic methodology of selecting aptamers[22,76]. For therapeutic uses, Pegaptanib (Macugen) held the patent and was the first aptamer-based drug approved by the FDA. The patents cover the aptamer and its use in treating the disease[4].

A patent was filed by inventor Caris Life Sciences Switzerland Holdings, S. A. R. L., Basel (CH) for developing aptamers and aptamer pools that bind relevant

biomarkers like microvesicle surface antigens or functional segments of microvesicle surface antigens and their methods[28]. This invention relates to new compounds based on nucleotides to prepare compounds that are radiolabelled and such compounds are used for diagnostic imaging. In compliance with the Indian Patents Act, 1970, an invention entitled aptamer against M.Tb HUPB and use thereof was awarded for the generation of DNA aptamers against HupB, a protein essential to the biology of Mycobacterium tuberculosis (MTb). The vital histone-like protein HupB (Rv2986c) plays a role in the entry and survival of MTb within host cells.

In a patent, pathogen detection is performed with Aptamer Molecular Beacons using a mobile device in which, after attaching to the target molecular sequence and being activated by the smartphone's flash, the Aptamer Molecular Photonic BeaconTM (AMPB) begins to emit photons. The AMPBs emit light at a different wavelength from the flashlight, making them easily distinguishable. Viruses and pathogens are identified using the smartphone's camera sensors[48].

"Dual Checkpoint Inhibitor Aptamer Based Therapeutics" is the title of a provisional patent application that Regen BioPharma Inc. submitted to the USPTO. The application deals with innovative compositions that can function as traditional checkpoint inhibitor treatments while also silencing genes like NR2F6 and Survivin regulating T cells and cancer cells. This technology is used to combine gene silencing with immunotherapy in a single therapy.

DNA aptamer, AS1411 is designed to target nucleolin, a protein prevalent in certain cancer cells. Patents related to AS1411 cover its structure and its use in cancer therapy[51]. Spiegelmers are mirror-image aptamers that bind to the enantiomers of natural amino acids. They have enhanced stability *in vivo* and are resistant to nuclease degradation. Patents in this category cover the structure and applications of these modified aptamers.

Some of the recent patents in the area of aptamers are given in table 1.

Somalogic Recent patents: SomaLogic Inc. is a clinical diagnostics and protein biomarker development firm that analyzes changes in protein content in biological samples to track health and disease. They created the SomaScan that enables researchers and scientists to find protein biomarkers for illnesses and ailments and use them in the development of new drugs and diagnostics. As per the information mentioned on the website of SomaLogic, there are 332 domestic and foreign pending patent applications and 601 domestic and abroad issued patents are owned by SomaLogic. Some of its recent patent granted are described in the table 2.

With the rise of computational biology, there are patents related to databases that store aptamer sequences and their targets. Additionally, software tools designed to predict aptamer-target interactions or to aid in aptamer design might also be patented. The patent landscape for aptamers is vast and multifaceted, reflecting the versatility of these molecules. As aptamers continue to be explored for new applications, it is anticipated that the patent filing will expand further.

Aptamer database: The growing interest in aptamers and their diverse applications has necessitated the generation of databases to systematically catalogue and provide access to the wealth of information related to aptamer sequences, structures and targets. These databases serve as repositories that can be utilized by researchers to study existing aptamers, design new ones, or even understand interactions between aptamers and their respective targets. An overview of existing aptamer databases is mentioned in table 3.

Beyond simply serving as repositories, these databases often come with functionalities that facilitate data analysis. This could be in the form of tools to compare sequences, to predict secondary structures, or to even model binding affinities. Such databases not only centralize the wealth of knowledge about aptamers but also foster collaboration among researchers. By accessing a database, researchers can build upon existing knowledge, avoid potential pitfalls and expedite the development of novel aptamers. The database classifies targets such as proteins, peptides, small molecules and cells, offering comprehensive information on each. This resource is particularly useful for researcher's keen on studying the breadth of aptamer applicability or wanting to target a specific molecule[18].

Table 1
Recent Patents in the area of Aptamer

Year	Patent No.	Title	Inventors
2023	US 11,806,419 B2	Method for Personal care composition comprising an aptamer composition comprising at least one oligonucleotide composed of nucleotides selected from the group consisting of: deoxyribonucleotides, ribonucleotides, derivatives of deoxyribonucleotides, derivatives of ribonucleotides and mixtures thereof[46].	(Juan Esteban Velasquez, Amy Violet Trejo, Gregory Allen Penner, Stevan David Jones); The Procter and Gamble Company

2023	US 11,579,110 B2	Aptamer-based sensors for detection of fentanyl opioids[86].	Yi Xiao, Zuan Canoura
2022	US 11,408,850 B2	Aptamer-based sensors for detection of fentanyl opioids[86].	Yi Xiao, Zuan Canoura
2021	US 11,060,095 B2	Method for isolating cross-reactive aptamer and use thereof[81].	Weijian Yang, Haixiang Yu, Yingzhu Liu, Yi Xiao
2021	US 10,907,163 B1	Aptamers that bind to natural and synthetic cannabinoids[85].	Yi Xiao, Haixiang Yu
2021	US 10,907,162 B2	Method for isolating cross-reactive aptamer and use thereof[81].	Weijian Yang, Haixiang Yu, Yingzhu Liu, Yi Xiao
2021	EP3 981 878 A1	FPR2 receptor agonist aptamers and uses thereof[14].	Carretero Trillo, Marta, De Arriba Pérez, María Del Carmen Del Río Nechaevsky, Marcela Andrea Fernández Gómez-Chacón, Gerónimo González Muñoz, Víctor Manuel Carrión Marchante, Rebeca Martín Palma, Elena
2021	US 10,927,404 B1	Pathogen Detection Using Aptamer Molecular Beacons Using a Mobile Device[48].	Najeeb Khalid
2020	EP 3 960 862 A1	FPR2 receptor agonist aptamers and uses thereof[14].	Flachbart, Lion 40597 Düsseldorf (DE) • Mahr, Regina 50226 Frechen (DE) • Schaumann, George
2020	US 10,655,132 B1	Method for isolating cross-reactive aptamer and use thereof[81].	Weijian Yang, Haixiang Yu, Yingzhu Liu, Yi Xiao
2019	US 2019/0262383 A1	CCR7 aptamers and uses thereof[45].	John Rossi, Azusa; Jiehua Zhou, Monrovia; Mayumi Takahashi Pasadena
2018	US-9958448-B2	Aptamers and uses thereof[29].	Halbert david (us) Domenyuk valeriy (us) Spetzler david (us) Hornung tassilo (us) Schafer frank (de) Xiao nianqing (us) Caris life sciences switzerland holdings GMBH
2018	US 2018/0346912 A1	Method for stabilizing DNA aptamers[41].	Ichiro HIRAO; Michiko HIRAO; Kenichiro MATSUNAGA
2017	US 9644202 B2	Switchable aptamer[8].	Berezovski, Maxim V. (Ottawa, CA) Wehbe, Mohamed (Ottawa, CA) Labib, Mahmoud Aziz Mahmoud (Ottawa, CA) Muharemagic, Darija (Gatineau, CA) Zamay, Anna S. (Krasnoyarsk, RU) Ghobadloo, Shahrokh (Ottawa, CA
2017	WO2017048147	The method of synthesis and purification of a nucleoside and/or a nucleotide, a modified nucleoside and/or nucleotide, a DNA molecule and an oligonucleotide library comprising said modified nucleoside and/or nucleotide and the use of said oligonucleotide library[47].	Jurek, Przemysław Jeleń, Filip Mazurek, Maciej Jakimowicz, Piotr (Pure Biologics Spółka Akcyjna
2017	EP3266871B1	Method for stabilizing DNA aptamers[41].	Hirao Ichiro [Jp]; Hirao Michiko [Jp]; Matsunaga Kenichiro [Jp]
2017	EP 3 369 819 B1	DNA aptamer binding to cancer cell[25].	FUTAMI Kazunobu Tokyo 1530041 (JP) • HIRAO Ichiro Tokyo 1530041 (JP) • HIRAO Michiko Tokyo 1530041 (JP)

Table 2
Somalogic patents in the Year 2023[39]

Year	Patent No.	Title
2023	US20230313202A1	Oligonucleotides comprising modified nucleosides
2023	US20230213502A1	Next-generation sequencing for protein measurement
2023	US20230193287A1	Nucleic acid compounds for binding growth differentiation factor 11
2023	US20230191414A1	Method for conducting uniform reactions
2023	US20230093170A1	Nucleic acid compounds for binding to complement component 3 protein
2023	US20230071234A1	Nonalcoholic steatohepatitis (NASH) biomarkers and uses thereof
2023	US20230048910A1	Methods of determining impaired glucose tolerance

Table 3
Few of the aptamer databases

S.N.	Database	Description	Website
1.	RNAapt3D	Unknown structures of RNA aptamer and its target moiety along with the complexes formed can be predicted using an RNA aptamer with 3D structural modeling library[69].	https://rnaapt3d.medals.jp.
2.	UTexas Aptamer Database	A searchable database containing aptamer data acquired from 1990–2022 and an aptamer dataset that is available to the public. Aptamer sequences, binding and selection data are included in the dataset with 1,443 reviewed aptamer records[6].	https://sites.utexas.edu/aptamerdatabase
3.	AptaDB	Database with aptamer-target interactions, binding affinity values and aptamer sequences that are validated experimentally. It contains information about: 1.information about the aptamer-target interaction that has been experimentally validated 2. information about aptamer properties, 3. information about the aptamer's structure, 4. information about targets, 5. information about experimental activity, 6. information about algorithmically calculated similar aptamers[16].	http://lmmd.ecust.edu.cn/aptadb/
4.	Aptabase	Includes plugins to estimate the GC content present in the DNA and RNA aptamers and it has aptamer data from over 600 aptamers.	https://www.iitg.ac.in/proj/aptabase/about.html
5.	Apta-Index	Aptagen's database containing data from published publications[5].	https://www.aptagen.com/apta-index/

Keeping the database updated with the rapidly expanding literature on aptamers is a significant challenge. The integration of ML and AI could aid in the automated extraction and classification of information. Future databases may offer more interactive tools like *in silico* aptamer design, predictive modelling of aptamer-target interactions, or even virtual screening platforms. The emergence and maintenance of aptamer databases underscore the scientific community's recognition of the significance of these molecules. As the field of aptamer research continues to expand, these databases will play an increasingly central role, streamline efforts and foster collaboration.

Traditional method of aptamer development and its limitations

SELEX (Systematic Evolution of Ligands by EXponential enrichment), is a process of generation of aptamers. This method is presently used as a standard technique to separate high-affinity single-stranded (ss) DNAs or RNAs from a huge library of random sequences. Aptamers are SELEX-derived DNAs and RNAs that can be selected against a wide variety of targets including toxins, chemicals, bacteria and viruses. Such generated aptamers filter down a large pool of randomly generated sequences until only a small number of sequences with the desired characteristics remain. This method involves amplification of specific sequences by

polymerase chain reaction (PCR) and an iterative *in vitro* selection process for the purpose of screening the target oligonucleotide probes from huge libraries of oligonucleotides.

The traditional SELEX approach uses ssDNA or ssRNA molecules with a random central region of 20-100 nucleotides to create a synthetic library. The 5' and 3' end of the oligonucleotide ends are flanked by specific sequences that act as the target for primers for PCR amplification during the selection stages.

The pool typically contains nearly 10^{15} different molecules providing a diverse range of potential configurations to choose the aptamers with the high affinity for a ligand. SELEX generates aptamer-protein complexes by starting with a combination of the target and library. Following the removal of non-specific sequences, potential aptamers are eluted for PCR amplification. At the end of each cycle, double-stranded amplicons are separated to create a new library with progressively more specific candidates. The aptamers undergo cloning and sequencing for their final modeling and characterisation following large selection rounds, typically 15-25 rounds.[17] The steps of SELEX are presented in fig. 1.

Limitations of SELEX method

The high expenditures of this process, the restriction of polymerase activity on the modified and non-natural nucleic acids and the shortened half-life *in vivo* due to nuclease degradation are few of more factors that have led to a decrease in the generation of aptamers by SELEX method.

Immobilization matrix: The immobilization of the target molecule on a suitable matrix is crucial to the SELEX procedure. A nitrocellulose filter is the most fundamental matrix used for immobilization of the target. It is inexpensive, easy to use, but works mostly for big molecules like proteins or cells due to its large pore size. Small compounds can be coupled to the matrix in bead-based SELEX process. The aptamers may experience steric hindrance from the matrix, which might decrease or prevent binding, particularly when small molecules are involved[68].

Constant domains: When the random region in the aptamer is 30 nt or shorter in length, primer-binding sites are more likely to have an impact on the aptamer structure. Additionally, the diversity of the structure in the library may be reduced if the constant regions form stable structures. The impact of the conserved regions on the structure of aptamer decreases with increasing length of the random region. The constant region's low complexity restricts structural diversity; hence, binding motifs are more likely to be located in longer, consequently more complex, random regions. In the longer sequences, the constant domain is less accessible, found near the oligonucleotide ends. Constant regions in aptamer structures prevent primer binding, which might cause complications during amplification[52].

PCR bias: PCR bias is inevitable during the amplification of an oligonucleotide library with high heterogeneity. PCR typically favors short sequences, which are amplified more quickly than lengthy sequences. Undesirable by-products like product–product, product–primer and even primer–primer hybridizations, may be produced as a result of the unspecific interactions. High structural stability aptamers, particularly those abundant in G and C bases, are typically less accessible to primers and are overpowered by lower structural stability aptamers[66].

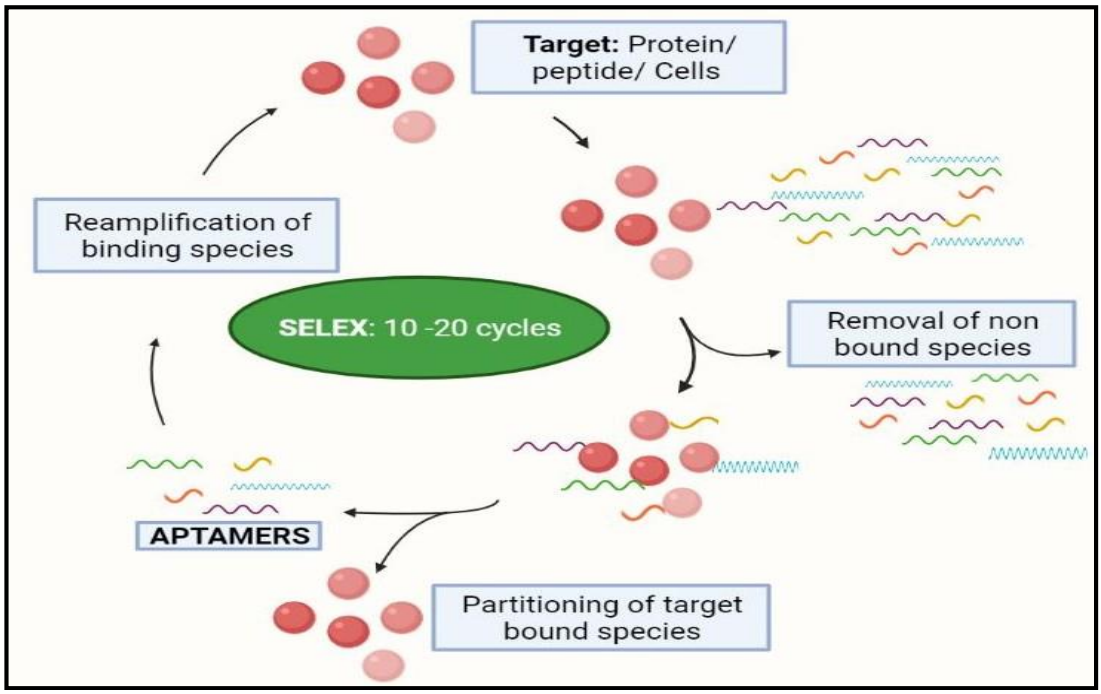

Fig. 1: A typical SELEX process

Purification: Most of the time, the availability of purified target molecules is necessary for aptamer generation. Due to their chemical composition, certain proteins can be difficult to purify. Due to the post-translational changes, aptamers produced against target proteins expressed in prokaryotic cells may not interact with the identical proteins expressed in eukaryotic cells due to restriction to access the epitopes of eukaryotic proteins due to these changes[57].

Binding Affinity (Kd): The affinity of particular aptamers is a crucial SELEX criterion. Among the most frequently used approaches for measuring binding affinities are separation-based techniques, many of which are more challenging for smaller-molecule targets than for proteins. In particular, when the target is significantly smaller than the aptamer, separation-based techniques that depend on a change in the size of aptamer–target complex upon target binding, are not very effective[59].

Cross Reactivity: Aptamer interaction with other proteins may result in adverse effects, aptamer cross-reactivity may be a hurdle in the practical use. Aptamers may bind to molecules with a similar structure after recognizing certain targets.

High Time and Cost: The process of aptamer generation appears to be a very simple procedure, but in reality, it takes a lot of time and effort. In order to create a sizable DNA oligonucleotide library, at least 10^{15} sequences are required to begin a selection procedure. The expense of choosing an aptamer is significantly increased since a robotic station for chemical synthesis must be installed in a lab for this purpose. The amplified aptamers are then subjected to several additional rounds in the process which may typically be around 15-25 rounds. With each successive round, the conditions are often made more stringent, promoting the selection of sequences with the highest affinity for the target.

Owing to the limitations discussed above, many advanced computational tools and algorithms have been developed that are simple to use, cost-effective, time-efficient and do not require specialized resources.

Computational approach using AI/ML

As an effective and economical alternative to traditional SELEX screening, the *in silico* aptamer screening uses computational simulation and molecular docking techniques to predict and screen candidate aptamer sequences that interact with certain proteins. Various tools, dedicated software programs and web servers have been designed to determine the structural and physicochemical features of aptamer sequences and their tendency to bind to their target. These tools encompass two primary categories of aptamer computational prediction techniques - interaction-based prediction and structure-based prediction[23].

Interaction-based prediction and structure-based prediction are the two primary categories of computational prediction methods for the aptamer. The physicochemical, kinetic and structural properties of the aptamer serve as the foundation for interaction-based computational prediction models. These tools shed light on the science underlying how aptamers and their targets interact. The computational prediction models based on structural folding are more accurate, but their application depends on the availability of the homologous sequence of the aptamer[78].

Entire process for aptamer selection and designing i.e. aptamer design, aptamer protein binding prediction, aptamer modeling and aptasensor design are all made possible by computational and artificial intelligence technologies as outlined in fig. 2. Table 4 of computational and AI based tools has been compiled based on the type of function for aptamer development.

Table 4
Computational and AI based tools have been compiled based on the type of function for aptamer development.

S.N.	Name of the tool	Function/Purpose	Based on Technique/ Platform
		Aptamer Selection/ Identification	
1.	AIoptamer	This tool accelerates up aptamer design and discovery by combining AI with traditional computational techniques.	Based on Structural modeling through CHIMERA_NA, an in-house mutagenesis tool
2.	AptaGPT	To design and optimize aptamers for generating potential high-affinity aptamer sequences	Generative Pre-trained Transformer (GPT) model
3.	PTANI	Computer program that uses sequence-structure motif analysis of HT-SELEX data to choose aptamers.	Built on the AptaMotif method with the implemenation of the potential binding motifs and the aptamers with the highest binding probability through the of integrative analysis of frequency and secondary structure.
4.	APTANI 2	This tool uses sequence-structure analysis to select target-specific aptamers from high-throughput SELEX data, ranks aptamers and recognizes significant structural motifs by calculating a score after combining the	Built on the AptaMotif method.

		frequency and structural stability of all predicted secondary structure of unique aptamer sequence. Implemenation of the potential binding motifs and the aptamers with the highest binding probability through the of integrative analysis of frequency and secondary structure.	
5.	DeepAptamer	It is used to identify high affinity sequences from the unenriched early SELEX rounds	It is based on bidirectional long short-term memory and hybrid neural network model combining convolutional neural networks
6.	RNAGEN	Short RNA sequences having natural RNA-like characteristics i.e., the secondary structure and free energy, can be generated via this approach. These sequences are fine-tuned for binding to a target protein using an optimization method. The model was guided by RNA-protein binding predictions found in the literature. It was discovered that this model can be trained for proteins that are similar, like proteins of similar nature, to generate a binding RNA molecule for the target protein even in the absence of a guide model built specially for the target protein.	This method is based on generative adversarial networks (GAN)
7.	RaptGen	RaptGen is an aptamer generating variational autoencoder (VAE). It effectively creates latent space where sequences cluster as per the motif structure using a profile HMM decoder. Those aptamers were generated that were not present in the highthroughput sequencing data using the latent r epresentation. Additionally, the methods for activity-guided aptamer synthesis and sequence shortening are also suggested.	RaptGen efficiently represents motif sequences by using of a profile hidden Markov model decoder.
8.	RaptRanker	It is an RNA aptamer selection tool analyzing the nucleotide sequence and its secondary structure from HT-SELEX data.	Based on C++ compiler, C Make and Boost Libraries
9.	Apta-MCTS	Using Apta-MCTS, this technique can produce the aptamer sequences against the target proteins. It then uses a docking simulation tool to combine the aptamer with the structures of target protein and assess our Apta-MCTS based on the simulation's docking scores. This verification study demonstrates that Apta-MCTS may effectively produce possible candidate aptamer sequences *in silico.*	A model that utilizes the Monte Carlo tree search (MCTS) technique
10.	AptaDiff	Aptadiff can generate aptamers that are not dependant on high- throughput sequencing data by using latent embeddings that are motif-dependent from variational autoencoders. It can also optimize the aptamers through aptamer synthesis that is affinity- guided as per the Bayesian optimization. Comparative analyses of four high-throughput screening data sets targe ting different proteins demonstrated AptaDiff's advantages over the current aptamer generation techniques in terms quality	Based on the diffusion model that enables *in silico* aptamer design and optimization
11.	UltraSelex	UltraSelex is a noniterative technique that identifies RNA aptamers in roughly a single day by combining computational signal-to-background rank modeling, high-throughput sequencing and biochemical partitioning.	This technique combines wet-lab partitioning with statistical rank modeling for aptamer discovery
12.	Aptamotif	Used to identify RNA binding motifs in sequence structure in aptamers produced from SELEX.	Linux/Mac OS

13.	AptCompare	An automated tool for HTS-SELEX sequencing data pre-processing and analysis. The tool uses a meta-rank methodology to choose the best aptamer targets after assessing the performance of six popular aptamer motif discovery algorithms.	Linux/Mac OS/Windows/Galaxy
14.	FASTAptamer	Applications of FASTAptamer include aptamer selection, *in vivo* mutagenesis, several surface display techniques like peptide, antibody fragment etc., ribozyme or deoxyribozyme selections- requiring next-generation DNA sequencing.	Based on Modular Perl scripts compatible for all UNIX-like systems (having Linux and Mac OS X, including Perl interpreters)
RNA Secondary structure prediction			
15.	KNetfold	This software can predict the consensus RNA secondary structure for a specific nucleotide sequence alignment.	It is based on ML method (a network with k-nearest neighbor classifiers
16.	SPOT-RNA	Useful for improving the sequence alignment, functional annotations and modeling RNA structure.	Based on deep contextual learning mainly for base-pair prediction
17.	Apterion	Designing of aptamers that combines the ML principles with reinforcement learning and implementations of the classic tree search	Monte Carlo tree search (MCTS)
18.	Sequential Multidimensional Analysis algorithm	Used for aptamer discovery (SMART-Aptamer) obtained from high-throughput sequencing (HTS) data using SELEX libraries that are based on multilevel structure analysis and unsupervised ML inorder to discover nucleic acid recognition ligands having high accuracy and efficiency	Based on the MDA framework, comprising of three metrics, termed as K score, F score and S score
Aptamer Protein Binding prediction			
19.	Seq2Feartur	Seq2Feature is a web-based tool that calculates 252 protein and 41 DNA descriptors that include the physicochemical properties, conformational and structural nature of DNA and proteins and the nucleotide composition and contact potentials of these molecules can also be derived. This tool can let users extract protein and DNA sequence-based features for inputs in ML techniques.	Python-based toolkit
20.	AptaTrans	AptaTrans is a deep learning framework used for prediction of aptamer-protein interaction (API) that computes the matrix of interaction between aptamers and proteins at the level of monomer. The encoder was pretrained using self-supervised learning techniques that make use of the secondary structures of the molecules and the predictions of masked tokens in order to guarantee the best sequence embeddings. Standard benchmark datasets that are frequently used for API prediction were used to assess the AptaTrans model's effectiveness. When compared to current data mining and ML techniques, the model performed better.	AptaTrans handles sequences of aptamer and protein at the monomer level using transformer-based encoders.
21.	Making Aptamers without SELEX (MAWS)	An algorithm that generates aptamers against target molecules and doesn't require an initial aptamer sequence or pool. The nucleotide with the lowest entropy is then selected as the starting point for the first sampling, which involves sampling nucleotides from a uniform distribution and docking. The sequence with the lowest energy is then selected by sampling potential conformations of the subsequent nucleotide in the sequence	-
22.	AptaLoop	This repository comprises the code for aptamer designing. *In silico* that was developed by the DTU Biobuilders 2023 iGEM team. There are four distinct	It is based on Python and uses mpmath for accurate floating-point computations and the open MM

		modules in the AptaLoop pipeline: 1. Prediction of secondary/tertiary structures 2. Making Aptamers (MAWS) Without Selex 3. Docking 4. Dynamics of Molecular Structure	package for molecular dynamics simulations.
23.	MP Bind	Statistical framework based on meta-motifs that is used for prediction of aptamers binding to targets using data of SELEX-Seq and effectively handling biases due to aptamer pool partial sequencing.	Linux/Mac OS
Tools for multiple functions of Aptamer development and selection			
24.	PATTERNITY-Seq©	This tool regroups the aptamer sequences into families, tracks the formation of each sequence and each family during the SELEX process, finds structure motifs that are enriched and analyzes the results of selection pressure. It does this by managing millions of sequences from unprocessed sequencing data to find best aptamers quickly.	Linux/Mac OS/Windows
25.	AptaPLEX	A program developed for method of demultiplexing unprocessed data obtained from HT-SELEX into appropriate selection rounds categories with the help of barcode based information.	Linux/Mac OS/Windows
26.	AptaSIM	This tool was designed to use error-prone PCR to accurately replicate the selection process during SELEX.	Linux/Mac OS/Windows
27.	AptaMUT	This is a new method to detect polymerase errors that lead to a higher binding affinity over the original sequence. It helps in identifying mutations for improving binding affinity.	Based on enrichment having cycle-to-cycle information with a probability model for identification of binding-improving mutations
28.	AptaCLUSTER	This technique clusters Whole HT-SELEX aptamer pools efficiently.	Based locality-sensitive hashing which is a random dimensionality reduction technique.
29.	AptaTRACE	This computational method uses the experimental design and protocol of the HT-SELEX, secondary structure of RNA and the probable presence of numerous secondary motifs to find sequence-structure motifs that exhibit a signature of selection. The three parts of AptaTRACE are motif extraction, secondary structure profile prediction and data preparation.	Based on computational method of distribution of the confirmational frameworks of all probable k-mers (all nucleotides sequences of length k) in all aptamers
30.	AptaGUI	A novel, open-source graphical user interface (GUI) that runs on all platforms for dynamically visualizing HT-SELEX data. Applications of the AptaTools package, which includes useful algorithms for analysis of HT-SELEX, such as data preprocessing and monitoring the evolution of individual aptamers and the entire aptamer family throughout the selection cycles, are supported by AptaGUI.	It is a new freely available and platform-independent user interface for the dynamic visualization of data of HT-SELEX
31.	AptaSUITE	AptaSUITE includes a set of algorithms - AptaCLUSTER AptaPLEX and AptaTRACE AptaSIM.	AptaSuite is a set of meticulously designed application programming interfaces (APIs) and matching reference implementations that make it easier to input, output and manipulate aptamer data.
Tools for Aptasensor designing			
32.	MEME/GLAM	To develop aptasensors by identification of motifs in aptamers through MEME analysis by estimating the recurrent, fixed length patterns (motifs)—in the selected aptamers.	Linux/Mac OS/Web

Future of computational techniques and AI for the aptamer selection

The progress in aptamer research holds promise for developing novel aptamers against unidentified disease targets in conjunction with the novel AI-based computational tools to improve treatment efficacy, imaging and biosensing capabilities. To accelerate the aptamer discovery process and maximize performance, computational techniques like AI and ML will be essential for predicting aptamer structures, affinities and possible interactions.

Few computational methods can help in differentiating between selective and nonspecific binding sequences during the early stages of SELEX. Such methods might be used to create more accurate prediction models with more data which would ultimately cut down on the number of SELEX cycles required for consensus aptamer sequence identification. A new method was developed by Heredia, Roche-Lima and Parés-Matos using ML and Natural Language Processing (NLP) approaches, to detect aptamers and to distinguish between binding between aptamers from targets of DNA-binding sequences that are non-specific.

CountVectorizer, an NLP technique, was utilized to get the nucleotide sequences to retrieve information. Sequence information and data from the NLP approach were used to train four ML algorithms: Gaussian naïve bayes, decision trees, logistic regression and support vector machines. Support vector machines, which distinguish between positive and negative classes, performed the best, with an accuracy of 0.995 and an area under the Receiving Operating Curve of 0.998. The developed AI method helps to minimize the number of rounds in a SELEX selection for identifying probable DNA aptamers. By removing non-specific binding sequences, this novel method might make it possible to pre-select DNA sequences from the first round of SELEX as probable aptamers for the second round of SELEX. These comparative studies could be used to create new screening libraries that purposefully exclude features that are not required of a DNA aptamer.

Aptamers can be detected more quickly and accurately with this method, which will enable the generation of more accurate aptamers in the future[31]. Quantifying the aptamer fitness, which influences the evolution of the sequence landscape at each selection round, is a challenge posed in the analysis of SELEX studies. Several methods to determine the fitness of a broad class of related sequences or a small number of chosen sequences have already been put forward including those based on *in silico* molecular dynamics simulations, sequence space clustering in conjunction with enrichment measures and direct fitness estimation investigations.

Di Gioacchino et al[20] have demonstrated the fitness of the experimentally selected sequence which can be directly correlated with the sequence probability assigned by the Restricted Boltzmann Machines (RBM). They have developed unsupervised learning method that uses RBM technique to model sequence ensembles derived from selection experiments. In order to identify thrombin bivalent aptamer nanostructures that bind two distinct exosites, the method was applied to previously acquired data from the SELEX experiment. RBMs might be helpful for modeling, may be in competition assays, where aptamers are selected to bind to a specific target such as cancer tissues and specifically do not bind to the healthy tissue. This method may be useful to develop more enriched sequence motifs that may get avoided in these best aptamers, as well as generate better or alternative binders for these intricate targets.

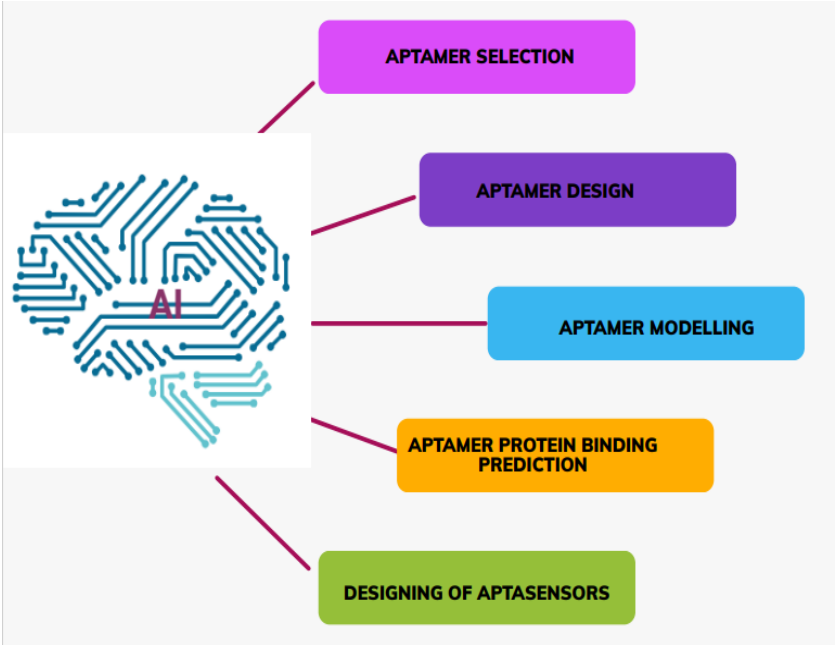

Fig. 2: Aptamer selection and designing approach using Artificial Intelligence

Modeling RNA and DNA regulatory sequences and its interaction with proteins in important processes like transcription regulation may be supported by this technique. Other selection-amplification techniques, like directed protein evolution research or phage display for antibody development which have a far wider range of potential sequences than aptamers. The aptamer libraries may be limited to a portion of the theoretical sequence space restricting the identification of certain aptamer sequences with favourable binding characteristics by scanning vast libraries. Identifying high-performing aptamers through this space can be achieved using ML features.

Bashir et al[7] have devised a new method to improve current experimental aptamer candidates for the protein neutrophil gelatinase associated lipocalin (NGAL) and have identified entirely new and shorter DNA aptamers to increase therapeutic efficacy. They created and validated an ML-guided particle display methodology (MLPD). By using this technique, they evaluated 187,499 aptamers experimentally and trained ML models to predict affinity to predict improvements to experimentally developed aptamers and to predict aptamers. They had also modified this strategy to instinctively identify candidates for producing short-length aptamers with high binding affinity. A desired target and undesirable homologues or protein mixture can be screened using this technique to design aptamers based on affinity and specificity.

Aptamers partition can be possible by various methods based on a few factors like nuclease stability or dissociation kinetics. The experimental optimization of multiparameter basis seems to be unachievable because of the combinatorial explosion of circumstances. There may be very little chance of designing an aptamer having the combination of a desired characteristic. However, the aptamer candidates having such parameters can be optimized *in silico* by converting experimental findings into N dimensional property vectors.[7]

The pharmacological and biosensing properties of aptamers are significantly influenced by their binding affinity to targets. For such a purpose, exact prediction of high-affinity aptamer modification techniques, even with the variety of post-SELEX alterations, has been considered for aptamer selection. In a study performed by Amu et al[3], a high-affinity aptamer modification technique for aptamers was developed using an interactive methodology integrating experimental findings and ML. An AI model with high prediction accuracy and a correlation coefficient value of 0.82 for the ones that were predicted and actual aptamer binding affinities were built following 04 rounds of interactive training using 422 modified aptamer-target affinity datasets with a variety of modification types and sites.

When compared to naturally unmodified aptamers, the ML - powered modified aptamer identified from this work demonstrated a 3.2-fold better Wnt-signal reactivation effect and a 105-fold higher affinity (picomole level KD value). This method predicted the most potential high-affinity modification strategy for aptamers by utilizing ML. Using a stacking learning technique, the model was trained in conjunction with Light Gradient Boosting Machine (LightGBM), Extreme Gradient Boosting (XGBoost) and Random Forest (RF). The DeepModify model significantly outperforms conventional techniques by offering a more effective, precise and reliable technique for predicting the post-SELEX modification binding affinity of aptamers. This breakthrough opens the door for the generation of high-affinity aptamers with improved probe sensitivity and therapeutic potential.[3]

A commonly used technique for numerical representation of the physicochemical and nucleotide composition of genomic data is the pseudo-K-tuple nucleotide composition method. By choosing different parameters and physicochemical qualities, users can quickly create a wide variety of PseKNC modes based on their needs. Nosrati and Amani[64] have developed a new ML technique for predicting the DNA aptamer for *E. coli* O157:H7, which includes the pseudo-K-tuple nucleotide composition approach. First, training data was generated using random DNA sequences and a validated aptamer set for the strains. The pseudo K-tuple nucleotide composition approach was used to transform the physico-chemical characteristics of the non-aptamer and aptamer sequences and nucleotide composition into numerical vectors.

Artificial neural network (ANN), random forests (RF) and support vector machine (SVM) algorithms were used to assess the potential of the prepared data for precise aptamer prediction. Lastly, the secondary structure and thermodynamics of the folding process were assessed for the screened aptamers using the exact algorithm. Despite the positive findings of the study, this approach's accuracy can be further increased by utilizing ensemble classifiers, expanding the training data and combining the outputs with an efficacy evaluator. Additionally, an *in vitro* analysis could bring out both benefits and drawbacks of the suggested approach[64].

Numerous new approaches are being researched for aptamer-target pair analysis and prediction. Sequence data from aptamers, as well as traditional and pseudo-amino acid composition of targets, all must be considered for developing a robust method for such prediction. In his study, Li et al[53] integrated features from aptamers and their targets in a novel approach for predicting aptamer-target interaction pairings. Aptamers and targets were represented by features of nucleotide and amino acid composition respectively as well as pseudo amino acid. The maximum relevance minimum redundancy (mRMR) and incremental feature selection (IFS) methods were used to select the best features for the predictor, which was built using Random Forest. The training dataset yielded 81.34% accuracy and 0.4612 MCC, while the testing dataset yielded 77.41% accuracy and 0.3717 MCC. 220 features were chosen as the

ideal feature set because they were thought to have made a substantial contribution to the predictions of the interacting aptamer-target pair. This prediction method may prove to be a helpful tool for identifying aptamer-target interaction. The identified and investigated features may offer valuable insights into the mechanism of interactions between aptamers and targets.

The available methods to measure the aptamer-target interaction prediction serve as classifiers while the techniques for analyzing data obtained from SELEX experiments are developed in the pool designing approach. However, generating novel aptamers for a variety of biomarkers is also crucial. In order to develop RNA aptamers *in silico*, a genetic algorithm (GA) with an incorporated binding predictor fitness function was used by Torkamanian-Afshar et al[75]. The entire process of creating an aptamer pool against the aminopeptidase N (CD13) biomarker was completed for this study. Initially, the model was created using the structural and sequential characteristics of well-known RNA–protein complexes.

Subsequently, novel aptamers were generated and top-ranked sequences were selected using RNA sequences involved in complexes with positive prediction results as the first generation. With a score three to six times greater than that of parent oligonucleotides, a 76-mer aptamer was found to have the highest fitness value. The reliability of the resulting sequences was verified through the molecular simulation and docking. This method proves to be a crucial and simple approach for the oligonucleotide–aptamer design process. This approach can be used for a variety of biomarkers in both therapeutic and diagnostic analysis. The selection of aptamers against a variety of biomarkers may be obtained using the methods used in the above mentioned work[75].

Few of the studies have focused on the initial library design based on the target secondary structure that proves to be an effective method for screening and optimization of aptamers. In a study by Chen et al[15], theophylline was used (1,3-dimethyl-7H-purine-2,6-dione) for aptamer complex as a case study to propose an aptamer design technique based on the target secondary structure of an existing RNA-ligand complex. In order to determine the vital bases that have been retained in the next sequence library design based on the target secondary structure, this two-step process started with molecular docking and molecular dynamics (MD) simulation of the initial aptamer-ligand complex. The binding free energy in MD was then used to conduct several rounds of virtual screening.

It is extremely difficult to search the whole sequence space for high-affinity aptamers for target molecules, both in virtual and experimental screening. A more effective aptamer library could be developed by combining the initial complex-based analysis with a secondary structure-based sequence library design technique. Compared to a library of entirely random sequences, an improved library design makes it simpler to find aptamers with high binding to target molecules. This approach works well for optimizing post-SELEX processes. If the binding conformation is suitable, one may identify aptamers by screening several times using this improved sequence library. The complex framework determines the point with the lowest energy as the simulation runs at various time scales, producing more insightful estimated results.

A significant improvement over conventional studies is the reduced screening time due to the improved library. The majority of sequences discovered more reasonable and stable binding sites during the screening process as the simulation's time scale increased and the binding energy remained more stable, demonstrating that this optimization technique can successfully find efficient aptamers.[15]

It is imperative to develop a model that distinguishes between sequences that are similar to the training set and those that are dissimilar, in order to generate new candidate sequences with a controllable degree of diversity. Moussa et al[61] have presented the Potts model, an unsupervised ML model to identify new sequences with controllable sequence diversity. Here, a specific feature that unifies training a Potts model with the maximum entropy principle on a limited number of sequences that have been empirically identified. Sequences that differ from the training set but are still likely to include the encoded features were produced by adjusting the Potts energy range that is sampled.

Using this method, different pools of sequences with particular secondary structural motifs were created for 30-mer RNA and DNA aptamers. This study provides a computational method for growing small datasets of aptamer sequences of DNA or RNA that possess a particular characteristic. This approach is suitable when test investigations are easy to conduct and are inexpensive. There is a high chance of discovering new and efficient sequences; sampling the Potts model's lowest energy region will increase the possibility of discovering valuable sequences. This novel computational method for enhancing the search of the chemical space of biopolymers with function and diversity in mind is based on unsupervised learning modeling.[61]

The revolutionary effects of AI technologies on Point -of - Contact (POC) biosensing, with a focus on current computational developments, solving persistent problems and potential future developments give a knowledge of the biosensing technologies and how they are used at the POC, emphasizing lingering problems and difficulties that AI could help with. AI in improving POC biosensing and educating scientists, medical professionals and decision-makers on how these technologies could change the world. AI in analyte selection, biomarker discovery, transduction, selecting recognition element selection, data acquisition, data handling and data analysis at every step of the biosensor

development process. Data interpretation is made possible by neural networks, ML algorithms and data processing frameworks that support analytical decision-making in real time[24].

Conclusion

Aptamers are used as diagnostic tools for identifying circulating tumor cells, biomarkers and pathogens and are now being designed for precision drug delivery, diagnosis of pathogens and have therapeutic applications for cancer and neurological disorders. The last few decades have seen significant development and advancement as a result of opportunities created by the emergence of advanced computational and AI technologies that have enabled overcoming the limits posed by these technologies. Advanced technologies like ML, computational modeling and AI could be combined with SELEX to speed up aptamer discovery and optimization.

Computational approaches, such as secondary and tertiary structure prediction, molecular dynamics simulations and molecular docking offer mechanistic understanding of aptamer-target binding. By learning from available aptamer databases, ML algorithms can develop small, diverse libraries, which help to minimize selection cycles. Aptamer selection is further refined by repeated feedback loops that combine experimental data and ML, turning the procedure into a data-driven pipeline that increases targeting precision and therapeutic potential in the complex biological frameworks.

Aptamer treatment is based on precision medicine approach which aims for personalized treatment and utilizes a more customized molecular approach and enhances pharmacogenomics to meet the demand for the appropriate medication at the appropriate time. The targets can then be fitted with drug-target interaction data to extract relevant medications and dosages from the derived data. However, their full therapeutic potential is limited by certain factors like structural instability, fast renal clearance and possible off-target effects. To overcome these constraints and improve their suitability in medical applications, ongoing research in chemical modification and delivery systems is vital.

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