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APTAMERS: A PROMISING NEW CLASS OF BIOSENSING OLIGONUCLEOTIDE THERAPEUTIC AGENT

***Arpan Adak, Sakasi Halder, Soumya Chakraborty, Dibya Dey, Surjya Toran Maiti and Dr. Dhrubo Jyoti Sen**

School of Pharmacy, Techno India University, Salt Lake City, Sector-V,EM-4, Kolkata-700091, West Bengal, India.

***Corresponding Author: Arpan Adak**

School of Pharmacy, Techno India University, Salt Lake City, Sector-V,EM-4, Kolkata-700091, West Bengal, India.

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ABSTRACT

Aptamers are a new class of biomolecules with a wide range of potential applications in medicine and other fields. Aptamers are short, single-stranded DNA or RNA oligonucleotides that can bind to a variety of targets with high affinity and specificity. Aptamers are generated through a process called systematic evolution of ligands by exponential enrichment (SELEX), in which a library of random oligonucleotides is repeatedly incubated with the target molecule and selected for binding. The oligonucleotides that bind to the target molecule are amplified and used to create a new library in the next round of SELEX. This process is repeated until a library of oligonucleotides with high affinity and specificity for the target molecule is obtained. Aptamers have several advantages over antibodies, including their ability to be generated against any type of target, their small size and ease of synthesis, and their stability in a variety of environments. Aptamers have a wide range of potential applications, including in diagnostics, therapeutics, drug delivery, and bio sensing. For example, aptamers can be used to develop new diagnostic tests for cancer, infectious diseases, and cardiovascular disease. They can also be used to develop new drugs and therapies for a variety of diseases like development of drugs to treat cancer, macular degeneration, and thrombosis. Aptamers are also being investigated for use in drug delivery and bio sensing. In drug delivery, aptamers can be used to deliver drugs and other therapeutic agents to specific cells and tissues. This can help to reduce the side effects of drugs and improve their efficacy. In bio sensing, aptamers can be used to develop biosensors that can detect a variety of molecules and organisms. This can be used to develop new diagnostic tests and to monitor the progress of diseases.

KEYWORDS: Aptamers, chemical antibodies, antibody mimics, biomolecular ligand.

ETYMOLOGY: The word "aptamer" is a neologism coined by Andrew Ellington and Jack Szostak in their first publication on the topic. They did not provide a precise definition, stating "We have termed these individual RNA sequences 'aptamers', from the Latin 'aptus', to fit."

Synonyms of aptamer: Nucleic acid aptamer**,** Oligonucleotide aptamer, DNA aptamer, RNA aptamer, Peptide aptamer, Chemical aptamer, Molecular recognition element, Affinity ligand, Aptamer sensor, Aptamer probe, Aptamer drug, Aptamer therapy, Theranostic.

Aptamer is an artificial chemical antibody that is generated from the randomized nucleic acid library by three simple steps: binding, separation, and amplification. The selected aptamer has a high binding affinity with the target molecule. Aptamers are occasionally classified as "chemical antibodies" or "antibody mimics". However, most aptamers are small, with a molecular weight of 6-30 kDa, in contrast to the 150 kDa size of antibodies, and contain one binding site rather than the two matching antigen binding regions of a typical antibody. Stable tertiary structure, resulting from combinations of these secondary structures, allows aptamers to bind to targets via van der Waals, hydrogen bonding, and electrostatic interactions. Aptamers are short, chemically synthesized, single-stranded RNA or DNA oligonucleotides that fold into unique three-dimensional structures, binding their target with structural recognition in a manner similar to an antibody–antigen interaction.[1]

History: Nobel laureate Jack Szostak [born November 9, 1952] and Andrew Ellington [March 15, 1767 – June 8, 1845] were among the inventors of SELEX and aptamers. Since its first application in 1967, directed evolution methodologies have been used to develop biomolecules with new properties and functions. Early examples include the modification of the bacteriophage Qbeta replication system and the generation of ribozymes with modified cleavage activity. In 1990, two teams independently developed and published SELEX

(Systematic Evolution of Ligands by Exponential enrichment) methods and generated RNA aptamers: the lab of Larry Gold, using the term SELEX for their process of selecting RNA ligands against T4 DNA polymerase and the lab of Jack Szostak, selecting RNA ligands against various organic dyes. Two years later, the Szostak lab and Gilead Sciences, acting independently of one another, used *in-vitro* selection schemes to generate DNA aptamers for organic dyes and human thrombin, respectively. In 2001, SELEX was automated by J. Colin Cox in the Ellington lab, reducing the duration of a weeks-long selection experiment to just three days.[2]

In 2002, two groups led by Ronald Breaker and Evgeny Nudler published the first definitive evidence for a riboswitch, a nucleic acid-based genetic regulatory element, the existence of which had previously been suspected. Riboswitches possess similar molecular recognition properties to aptamers. This discovery added support to the RNA World hypothesis, a postulated stage in time in the origin of life on Earth.

In this review, we will introduce the principle and workflow of variations of SELEX-based methods, including secretome SELEX, ADAPT, Cell-SELEX and tissue SELEX. Aptamers, particularly RNA strands, are prone to quick degradation in biological media due to interactions with biomolecules. Many aptamers have been shown to degrade in blood as quickly as a few minutes, which is far too short for most medical applications. Aptamer Group was founded in 2008 by Dr Arron Tolley, age 44, an early school leaver who later completed a doctorate in biophysics and molecular biology, and Dr David Bunka, a geneticist.[3]

Figure 1: (from left) Dr. Jack Szostak, Dr. Andrew Ellington, Dr. Arron Tolley, Dr. David Bunka.

Aptamers are biomolecular ligands composed of single-stranded (ss) nucleic acid (DNA or RNA) molecules that are specifically isolated from a random sequence pool via in vitro selection process known as Systematic Evolution of Ligands by Exponential Enrichment [SELEX]. Aptamers are single-stranded oligonucleotides that fold into defined architectures and bind to targets such as proteins. In binding proteins they often inhibit protein–protein interactions and thereby may elicit therapeutic effects such as antagonism half-life [5-10 minutes]. They are in general more stable than antibodies, and have a longer shelf life [2 years]. Aptamers are produced through a simple and inexpensive process and the time required to generate aptamers is comparatively short. Unlike antibodies, aptamers do not need animals or an immune response for their production. Aptamers are synthetic molecules that can be raised against any kind of target, including toxic or non-immunogenic ones. They bind their target with affinity similar or higher than antibodies. They are 10 fold smaller than antibodies and can be chemically-modified at will in a defined and precise way. Indeed, Aptagen now offer custom aptamers with prices ranging from $1–4 per microgram (for microgram quantities), $300 per gram (for milligram quantities), or even under $50 per gram (for gram quantities). Synthesis can be completed in two days or up to two weeks. Stored like this, most manufacturers guarantee a shelf life of 2 years. However, even at 37°C, it is estimated that the oligonucleotide would be stable for in excess of 6 weeks. Aptamers are developed through the process of systematic evolution of ligands by exponential enrichment (SELEX), which is repeated to increase the binding power and specificity. However, the SELEX process is time-consuming, and the characterization of aptamer candidates selected through it requires additional effort. Oligonucleotides are short nucleic acid polymers used in research, genetic testing and forensics. Oligonucleotides are usually made up of 13 to 25 nucleotides and are designed to hybridize specifically to DNA or RNA sequences.[4]

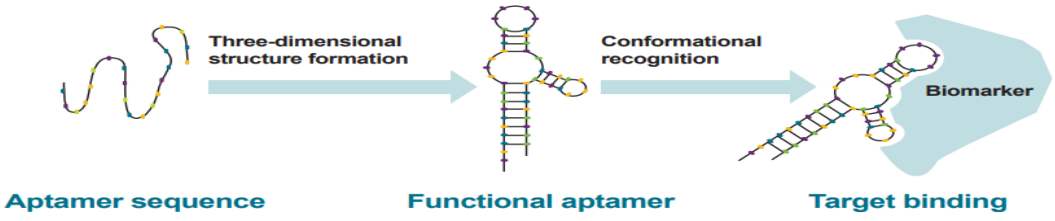

Figure 2: Aptamer formation.

Aptamers are short, single-stranded DNA or RNA (ssDNA or ssRNA) molecules that can selectively bind to a specific target, including proteins, peptides, carbohydrates, small molecules, toxins, and even live cells. Two major steps exist in aptamer design selection and optimization. In the first step, several polynucleotides with probable binding affinity toward a target are screened by using the SELEX method and then selected. In the second step, aptamers with detected high affinity are shortened, modified, and stabilized. Aptamers are short, single-stranded RNA (ssRNA) or DNA (ssDNA) molecules that assume various shapes, owing to their tendency to form helices or single-stranded loops. Indeed, several groups have successfully isolated aptamers capable of binding structured RNA molecules (12–16). Aptamer is an artificial chemical antibody that is generated from the randomized nucleic acid library by three simple steps: binding, separation, and amplification. The selected aptamer has a high binding affinity with the target molecule. First introduced in 1990 (Ellington and Szostak, 1990; Tuerk and Gold, 1990), aptamers are short synthetic single-stranded (ss) nucleic acid sequences that can bind to a broad range of targets including metal ions, chemical compounds, proteins, cells and whole micro-organisms. Bifunctional Aptamer–Doxorubicin conjugate crosses the Blood–Brain Barrier and selectively delivers its payload to EpCAM-Positive Tumor Cells.[5]

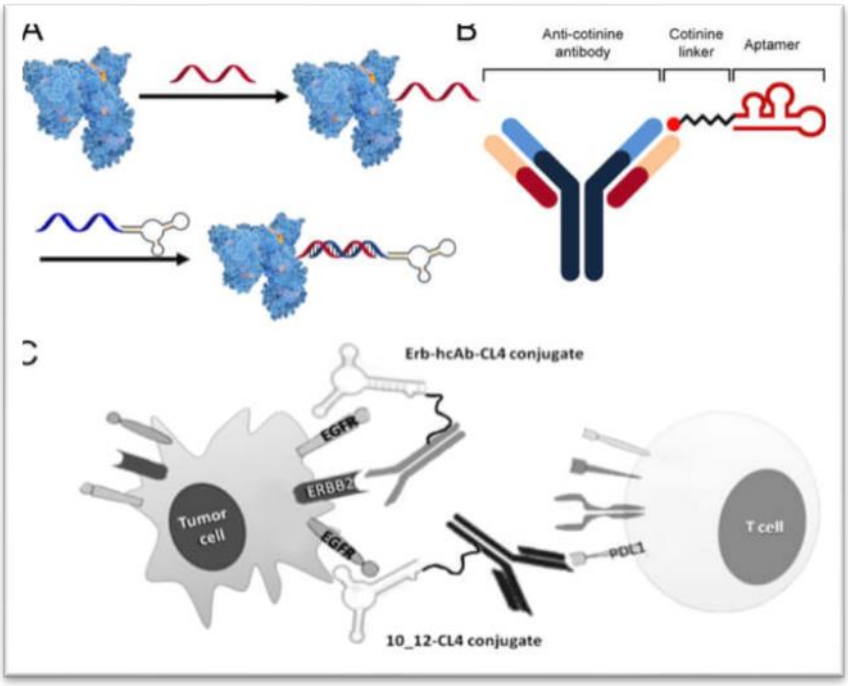

Figure 3: Aptamer-Drug Conjugate.

Aptamers are predicted to be highly useful in producing general drugs and theranostic drugs occasionally for certain diseases like cancer, Alzheimer's disease, and so on. Theranostics is a combination of the terms **thera**peutics and diag**nostics**. Theranostics is the term used to describe the combination of using one radioactive drug to identify (diagnose) and a second radioactive drug to deliver therapy to treat the main tumor and any metastatic tumors. They bind to various targets like lipids, nucleic acids, proteins, small organic compounds, and even entire organisms. Aptamer stability is a function of both structure and chemical composition. Tightly folded molecules are expected to be more resistant to degradation because of limited solvent exposure. Aptamers are the oligonucleotide chains with higher specificity and affinity towards target protein. Thus, aptamers are considered as a nucleic acid version of antibodies. Various antibodies mediated methods has adopted aptamer-based detection technology. Oligonucleotides are short nucleic acid polymers used in research, genetic testing and forensics. Oligonucleotides are usually made up of 13 to 25 nucleotides and are designed to hybridize specifically to DNA or RNA sequences.DNA is a very large molecule that contains millions of nucleotides! Oligonucleotides are short single or double-stranded fragments of DNA or RNA. In contrast to DNA, oligonucleotides usually contain from 13 to 25 nucleotides, but they can be larger as well. However, they rarely exceed 200 nucleotides. A good example of oligonucleotide use is as primers for PCR. Primers are single-stranded oligos required for initiation of DNA replication in PCR since DNA polymerases can only add nucleotides to existing nucleic acid sequence. In addition, aptamers are considerably larger than small molecules, which can lead to high signal/noise ratio in size-based measurements, thus only sensitive assays will be able to detect their interaction. Biosensors that are based on aptamers as biorecognition elements are named aptasensors. Aptamers used in the field of biosensors make use of their high affinity and tuneable properties, while their sensitivity is greatly influenced by the transducer.[6]

Aptamers are a class of targeting ligands that bind exclusively to biomarkers of interest. Aptamers have been identified as candidates for the construction of various smart systems for therapy, diagnosis, bioimaging, and drug delivery due to their high target affinity and specificity. There are 5 types of heavy chain constant regions in antibodies (immunoglobulin) and according to these types, they are classified into IgG, IgM, IgA, IgD, and IgE. They are distributed and function differently in the body.

Aptamer Folding / Dilution to Working Concentration

1. Prior to use, dilute the aptamer to 10 - 100x working concentration in Aptamer Folding Buffer
2. Heat the aptamer solution to 90-95°C for 5 minutes
3. Allow the aptamer solution to cool to room temperature (~15 minutes).

Real-time oligo stability depends on storage temperature and storage medium. Of these two variables, temperature is most important. When stored at –20°C (frozen), IDT oligos remain stable for two years (24 months), regardless of whether they are stored dry or re-suspended in TE buffer or nuclease-free water. Peptide aptamers are small combinatorial proteins that are selected to bind to specific sites on their target molecules. Peptide aptamers consist of short, 5-20 amino acid residues long sequences, typically embedded as a loop within a stable protein scaffold.[7]

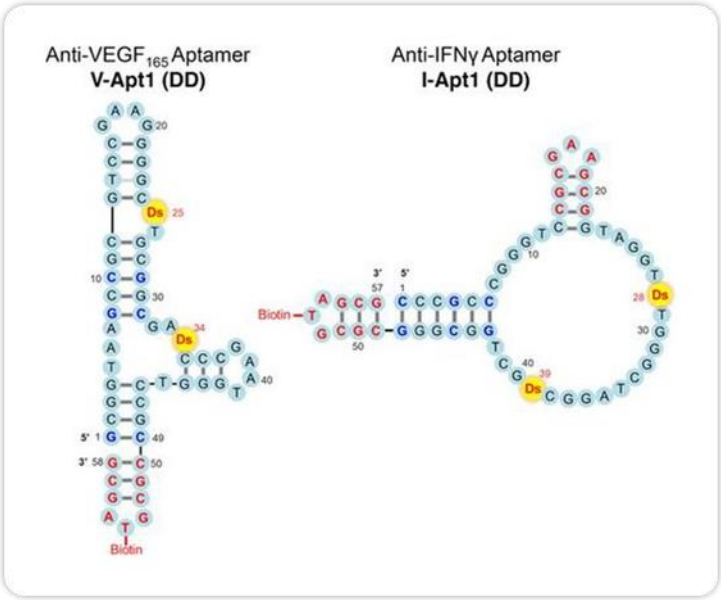

Figure 4: Aptamers.

Aptamers are single-stranded oligonucleotides that fold into defined architectures and bind to targets such as proteins. In binding proteins they often inhibit protein–protein interactions and thereby may elicit therapeutic effects such as antagonism. Like monoclonal antibodies, aptamers can be used for the molecular recognition of their respective targets. Aptamers have been successfully used for pathogen recognition, cancer recognition, monitoring environmental contamination, and as stem cell markers. Aptamers can be obtained through an iterative selection process known as SELEX (systematic evolution of ligands by exponential enrichment) by using single-stranded DNA or RNA. An initial pool of 1014-1015 random oligonucleotide (ONT) strands are subjected to binding with the target. Aptamers are oligonucleotides, such as ribonucleic acid (RNA) and single-strand deoxyribonucleic acid (ssDNA) or peptide molecules that can bind to their targets with high affinity and specificity due to their specific three-dimensional structures. Aptamers are a special class of nucleic acid molecules that are beginning to be investigated for clinical use. These small RNA/DNA molecules can form secondary and tertiary structures capable of specifically binding proteins or other cellular targets; they are essentially a chemical equivalent of antibodies. And as a multiplexed protein biomarker detection technology, aptamer-based SOMAScan can analyze thousands of proteins in a single run.[8]

Regardless of their high specificity, aptamers that recognize particular targets can also bind to molecules with a similar structure. Four aptamers against DNApolymerase β generated in our laboratory can also bind and inhibit DNA polymerase κ, which belongs to another DNA polymerase family. The resulting ligands were coined “aptamers” (derived from the Greek word aptus; “to fit”) by Andy Ellington and Jack Szostak in independent work that devised the same general strategy. Some aptamers exist naturally as ligand-binding elements, but most are generated *in-vitro* and tailored to specific purposes. A DNA aptamer is used in the design of an electrochemical ampicillin sensor. The sensor is reagent-less and can be reused for three times. The sensor's response to the target is fast. The sensor is

sensitive, specific, and can function well in complex samples. Aptamers, an emerging class of therapeutics, are DNA or RNA molecules that are selected to bind molecular targets that range from small organic compounds to large proteins. All of the determined structures of aptamers in complex with small molecule targets show that aptamers cage such ligands. Aptamers are nucleic acid-based ligands identified through a process of molecular evolution named SELEX (Systematic Evolution of Ligands by Exponential enrichment). Aptamers are short sequences of artificial DNA, RNA, XNA, or peptide that bind a specific target molecule, or family of target molecules. They exhibit a range of affinities (KD in the pM to μM range), with variable levels of off-target binding and are sometimes classified as chemical antibodies. Aptamers and antibodies can be used in many of the same applications, but the nucleic acid-based structure of aptamers, which are mostly oligonucleotides, is very different from the amino acid-based structure of antibodies, which are proteins. This difference can make aptamers a better choice than antibodies for some purposes. Aptamers are used in biological lab research and medical tests. If multiple aptamers are combined into a single assay, they can measure large numbers of different proteins in a sample. They can be used to identify molecular markers of disease, or can function as drugs, drug delivery systems and controlled drug release systems. They also find use in other molecular engineering tasks. Most aptamers originate from SELEX, a family of test-tube experiments for finding useful aptamers in a massive pool of different DNA sequences. This process is much like natural selection, directed evolution or artificial selection. In SELEX, the researcher repeatedly selects for the best aptamers from a starting DNA library made of about a quadrillion different randomly generated pieces of DNA or RNA. After SELEX, the researcher might mutate or change the chemistry of the aptamers and do another selection, or might use rational design processes to engineer improvements. Non-SELEX methods for discovering aptamers also exist. Researchers optimize aptamers to achieve a variety of beneficial features. The most important feature is specific and sensitive binding to the chosen target. When aptamers are exposed to bodily fluids, as in serum tests or aptamer therapeutics, it is often important for them to resist digestion by DNA- and RNA-destroying proteins. Therapeutic aptamers often must be modified to clear slowly from the body. Aptamers that change their shape dramatically when they bind their target are useful as molecular switches to turn a sensor on and off. Some aptamers are engineered to fit into a biosensor or in a test of a biological sample. It can be useful in some cases for the aptamer to accomplish a pre-defined level or speed of binding. As the yield of the synthesis used to produce known aptamers shrinks quickly for longer sequences, researchers often truncate aptamers to the minimal binding sequence to reduce the production cost.[9]

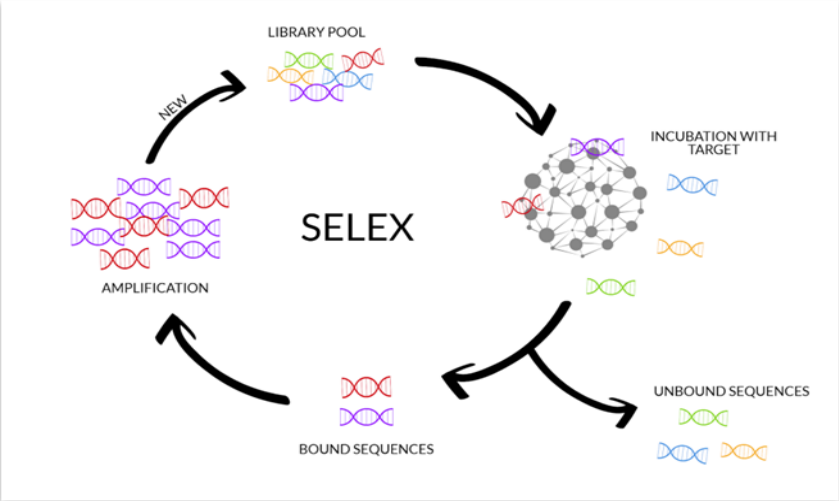

Figure 5: SELEX Cycle.

Classification: A typical aptamer is a synthetically generated ligand exploiting the combinatorial diversity of DNA, RNA, XNA, or peptide to achieve strong, specific binding for a particular target molecule or family of target molecules. Aptamers are occasionally classified as "chemical antibodies" or "antibody mimics". However, most aptamers are small, with a molecular weight of 6-30 kDa, in contrast to the 150 kDa size of antibodies, and contain one binding site rather than the two matching antigen binding regions of a typical antibody.

Typical aptamers can be classified into the following categories.

Based on sequence

- RNA aptamers
- DNA aptamers
- Peptide aptamers
- Chemical aptamers

Based on structure

- Stem-loop aptamers

- i-Motif aptamers
- Quadruplex aptamers
- G-quadruplex aptamers

Based on function

- Diagnostic aptamers
- Therapeutic aptamers
- Drug delivery aptamers
- Biosensor aptamers
- Theranostic aptamers

Diagnostic aptamers are used to detect the presence of specific molecules or cells in a sample. For example, diagnostic aptamers are being developed to detect cancer cells, infectious agents, and other biomarkers. Therapeutic aptamers are used to treat diseases by binding to specific targets and interfering with their function. For example, therapeutic aptamers are being developed to treat cancer, macular degeneration, and other conditions. Drug delivery aptamers are used to deliver drugs and other therapeutic agents to specific cells and tissues. This can help to improve the efficacy of drugs and reduce side effects.[10]

Biosensor aptamers are used to develop biosensors that can detect a variety of molecules and organisms. This can be useful for environmental monitoring, food safety, and other applications. Theranostic aptamers are aptamers that can be used for both diagnosis and treatment. For example, a theranostic aptamer could be used to detect cancer cells and then deliver a drug to kill those cells. It is important to note that these classifications are not mutually exclusive. For example, a therapeutic aptamer could also be used for diagnosis or drug delivery.

Here are some examples of typical aptamers

- RNA aptamers: Pegaptanib (Macugen), an aptamer that is used to treat macular degeneration. [RNA Aptamers are defined as **RNA oligonucleotides that bind to a specific target with high affinity and specificity**, similarly to how an antibody binds to an antigen.]
- DNA aptamers: Tenaplavoid (NOX-A1), an aptamer that is being developed to treat cancer. [DNA Aptamers are **short, single-stranded DNA molecules that can selectively bind to a specific target, including proteins, peptides, carbohydrates, small molecules, toxins, and even live cells.** Aptamers assume a variety of shapes due to their tendency to form helices and single-stranded loops.]
- Peptide aptamers: Cilengitide (Eligard), an aptamer that is used to treat prostate cancer. [Peptide aptamers consist of **short, 5-20 amino acid residues long sequences, typically embedded as a loop within a stable protein scaffold.**]
- Chemical aptamers: ARC155, a chemical aptamer that is being developed to treat COVID-19. [Chemical aptamers are **short, single-stranded oligonucleotides that bind to specific target molecules**. The shape-forming feature of single-stranded oligonucleotides provides high affinity and excellent specificity toward targets. Hence, aptamers can be used as analogs of antibodies.]

Properties

Structure: The complex and diverse secondary and tertiary structure of aptamers, as shown in this schematic of an aptamer's secondary structure, is what lets them bind their target strongly and specifically. Complementary base pairing is visible in the black lines connecting some G-C and A-T bases. This is a feature of nucleic acids that does not exist in the amino acids of antibodies. It helps aptamers form these unique structures. Hairpin regions (red), which rely on this base pairing, enhance the aptamer's stability at different temperatures. This image also shows examples of chemical modifications to the base aptamer. Two unnatural bases, which enhance durability, are in yellow. The biotin molecule binds with extreme strength to streptavidin, allowing the aptamer to be anchored to other molecules or to a surface in sensors and assays.[11]

Most aptamers are based on a specific oligomer sequence of 20-100 bases and 3-20 kDa. Some have chemical modifications for functional enhancements or compatibility with larger engineered molecular systems. DNA, RNA, XNA, and peptide aptamer chemistries can each offer distinct profiles in terms of shelf stability, durability in serum or in vivo, specificity and sensitivity, cost, ease of generation, amplification, and characterization, and familiarity to users. Typically, DNA- and RNA-based aptamers exhibit low immunogenicity, are amplifiable via Polymerase Chain Reaction (PCR), and have complex secondary structure and tertiary structure. DNA- and XNA-based aptamers exhibit superior shelf stability. XNA-based aptamers can introduce additional chemical diversity to increase binding affinity or greater durability in serum or in vivo.

As 22 genetically-encoded and over 500 naturally-occurring amino acids exist, peptide aptamers, as well as antibodies, have much greater potential combinatorial diversity per unit length relative to the 4 nucleic acids in DNA or RNA. Chemical modifications of nucleic acid bases or backbones increase the chemical diversity of standard nucleic acid bases. Split aptamers are composed of two or more DNA strands that are pieces of a larger parent aptamer that has been broken in two by a molecular nick. The ability of each component strand to bind targets will depend on the location of the nick, as well as the secondary structures of the daughter strands. The presence of a target molecule supports the DNA fragments joining together. This can be used as the basis for biosensors. Once assembled, the two separate DNA strands can be ligated into a single strand. Unmodified aptamers are cleared rapidly from the bloodstream, with a half-life of seconds to hours. This is mainly due to

nuclease degradation, which physically destroys the aptamers, as well as clearance by the kidneys, a result of the aptamer's low molecular weight and size. Several modifications, such as 2'-fluorine-substituted pyrimidines and polyethylene glycol (PEG) linkage, permit a serum half-life of days to weeks. PEGylation can add sufficient mass and size to prevent clearance by the kidneys in vivo. Unmodified aptamers can treat coagulation disorders. The problem of clearance and nuclease digestion is diminished when they are applied to the eye, where there is a lower concentration of nuclease and the rate of clearance is lower. Rapid clearance from serum can also be useful in some applications, such as in vivo diagnostic imaging.[12]

Targets: Aptamer targets can include small molecules and heavy metal ions, larger ligands such as proteins, and even whole cells. These targets include lysozyme, thrombin, human immunodeficiency virus trans-acting responsive element (HIV TAR), hemin, interferon γ, vascular endothelial growth factor (VEGF), prostate specific antigen (PSA), dopamine, and the non-classical oncogene, heat shock factor 1 (HSF1).Aptamers have been generated against cancer cells, prions, bacteria, and viruses. Viral targets of aptamers include influenza A and B viruses, Respiratory syncytial virus (RSV), SARS coronavirus (SARS-CoV) and SARS-CoV-2. Aptamers may be particularly useful for environmental science proteomics. Antibodies, like other proteins, are more difficult to sequence than nucleic acids. They are also costly to maintain and produce, and are at constant risk of contamination, as they are produced via cell culture or are harvested from animal serum. For this reason, researchers interested in little-studied proteins and species may find that companies will not produce, maintain, or adequately validate the quality of antibodies against their target of interest. By contrast, aptamers are simple to sequence and cost nothing to maintain, as their exact structure can be stored digitally and synthesized on demand. This may make them more economically feasible as research tools for underfunded biological research subjects. Aptamers exist for plant compounds, such as theophylline (found in tea) and abscisic acid (a plant immune hormone). An aptamer against a-amanitin (the toxin that causes lethal Amanita poisoning) has been developed, an example of an aptamer against a mushroom target. Aptamer applications can be roughly grouped into sensing, therapeutic, reagent production, and engineering categories. Sensing applications are important in environmental, biomedical, epidemiological, biosecurity, and basic research applications, where aptamers act as probes in assays, imaging methods, diagnostic assays, and biosensors. In therapeutic applications and precision medicine, aptamers can function as drugs, as targeted drug delivery vehicles, as controlled release mechanisms, and as reagents for drug discovery via high-throughput screening for small molecules and proteins. Aptamers have application for protein production monitoring, quality control, and purification. They can function in molecular engineering applications as a way to modify proteins, such as enhancing DNA polymerase to make PCR more reliable. Because the affinity of the aptamer also affects its dynamic range and limit of detection, aptamers with a lower affinity may be desirable when assaying high concentrations of a target molecule. Affinity chromatography also depends on the ability of the affinity reagent, such as an aptamer, to bind and release its target, and lower affinities may aid in the release of the target molecule. Hence, specific applications determine the useful range for aptamer affinity.

Antibody replacement: Aptamers can replace antibodies in many biotechnology applications. In laboratory research and clinical diagnostics, they can be used in aptamer-based versions of immunoassays including enzyme-linked immunosorbent assay (ELISA), western blot, immunohistochemistry (IHC), and flow cytometry. As therapeutics, they can function as agonists or antagonists of their ligand. While antibodies are a familiar technology with a well-developed market, aptamers are a relatively new technology to most researchers, and aptamers have been generated against only a fraction of important research targets. Unlike antibodies, unmodified aptamers are more susceptible to nuclease digestion in serum and renal clearance *in-vivo*. Aptamers are much smaller in size and mass than antibodies, which could be a relevant factor in choosing which is best suited for a given application. When aptamers are available for a particular application, their advantages over antibodies include potentially lower immunogenicity, greater replicability and lower cost, a greater level of control due to the *in-vitro* selection conditions, and capacity to be efficiently engineered for durability, specificity, and sensitivity. In addition, aptamers contribute to reduction of research animal use. While antibodies often rely on animals for initial discovery, as well as for production in the case of polyclonal antibodies, both the selection and production of aptamers is typically animal-free. However, phage display methods allow for selection of antibodies in vitro, followed by production from a monoclonal cell line, avoiding the use of animals entirely.[13]

Controlled release of therapeutics: The ability of aptamers to reversibly bind molecules such as proteins has generated increasing interest in using them to facilitate controlled release of therapeutic biomolecules, such as growth factors. This can be accomplished by tuning the binding strength to passively release the growth factors, along with active release via mechanisms such as hybridization of the aptamer with complementary oligonucleotides or unfolding of the aptamer due to cellular traction forces.

AptaBiD: AptaBiD (Aptamer-Facilitated Biomarker Discovery) is an aptamer-based method for biomarker discovery.

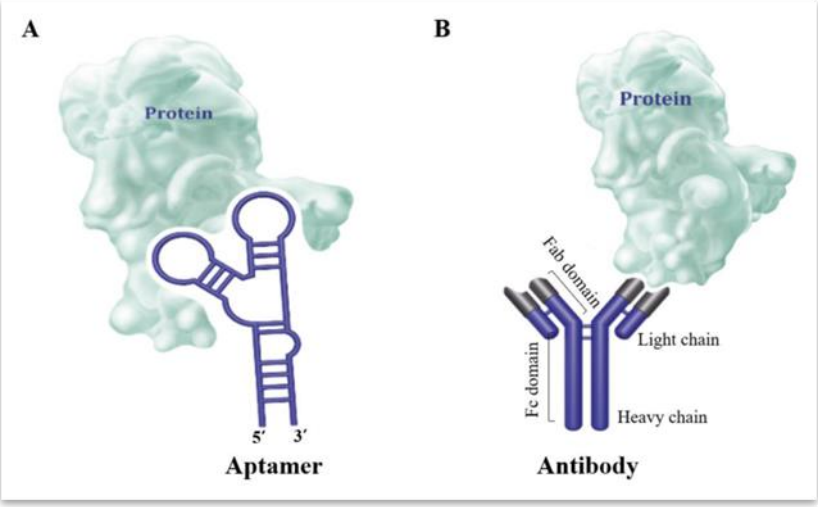

Figure 6: Difference between Aptamer and Antibody.

Peptide Aptamers: While most aptamers are based on DNA, RNA, or XNA, peptide aptamers are artificial proteins selected or engineered to bind specific target molecules.[14]

Structure: Peptide aptamers consist of one or more peptide loops of variable sequence displayed by a protein scaffold. Derivatives known as tadpoles, in which peptide aptamer "heads" are covalently linked to unique sequence double-stranded DNA "tails", allow quantification of scarce target molecules in mixtures by PCR (using, for example, the quantitative real-time polymerase chain reaction) of their DNA tails. The peptides that form the aptamer variable regions are synthesized as part of the same polypeptide chain as the scaffold and are constrained at their N and C termini by linkage to it. This double structural constraint decreases the diversity of the 3D structures that the variable regions can adopt, and this reduction in structural diversity lowers the entropic cost of molecular binding when interaction with the target causes the variable regions to adopt a uniform structure.

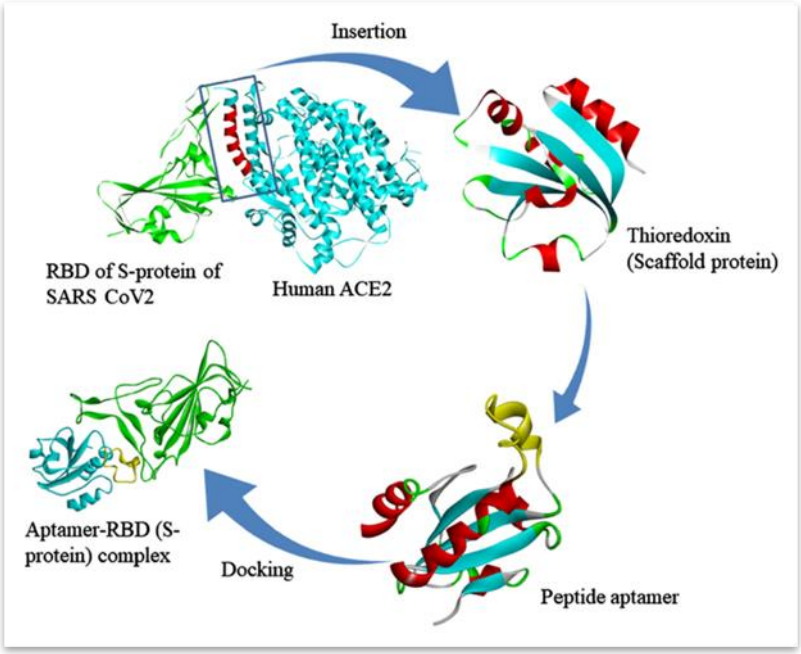

Figure 7: Designing of peptide aptamer targeting the receptor-binding domain of spike protein of SARS-CoV-2

Selection: The most common peptide aptamer selection system is the yeast two-hybrid system. Peptide aptamers can also be selected from combinatorial peptide libraries constructed by phage display and other surface display technologies such as mRNA display, ribosome display, bacterial display and yeast display. These experimental procedures are also known as biopanning. All the

peptides panned from combinatorial peptide libraries have been stored in the MimoDB database.[15]

Applications: Libraries of peptide aptamers have been used as "mutagens", in studies in which an investigator introduces a library that expresses different peptide aptamers into a cell population, selects for a desired phenotype, and identifies those aptamers that cause the phenotype. The investigator then uses those aptamers as baits, for example in yeast two-hybrid screens to identify the cellular proteins targeted by those aptamers. Such experiments identify particular proteins bound by the aptamers, and protein interactions that the aptamers disrupt, to cause the phenotype. In addition, peptide aptamers derivatized with appropriate functional moieties can cause specific post-translational modification of their target proteins, or change the subcellular localization of the targets. This assay tests the ability of two different types of aptamers (V and I) to detect their respective protein targets (VEGF and IFN-y). The labels Apt1, Apt2, Apt3, and Apt4 are in decreasing order of binding strength (i.e. Apt1 is the strongest aptamer). The DD, AD, DA, and AA letters mean that they have different combinations of unnatural base pairs. This causes their difference in binding strengths. The "-" columns have no protein, and the "+" columns do have protein. Aptamer with protein (+) and without protein (-) is loaded into wells in a gel and moves down the column lanes. If target is present, they bind and travel more slowly, due to the charge on the aptamer and the mass of the protein. Otherwise, the unbound aptamer moves quickly to the end of the lane. The difference in position between the "+" and "-" bands is the "mobility shift." This allows the researcher to detect the presence or absence of the protein. The darker band in the leftmost V and I lanes show that stronger aptamer-target binding makes it easier to detect the target at a given amount of target protein in the sample. The bottom image includes denaturing urea in the gel that disrupts aptamer-target binding in the weaker I aptamers, showing that the aptamer-protein binding is indeed what caused the mobility shift.

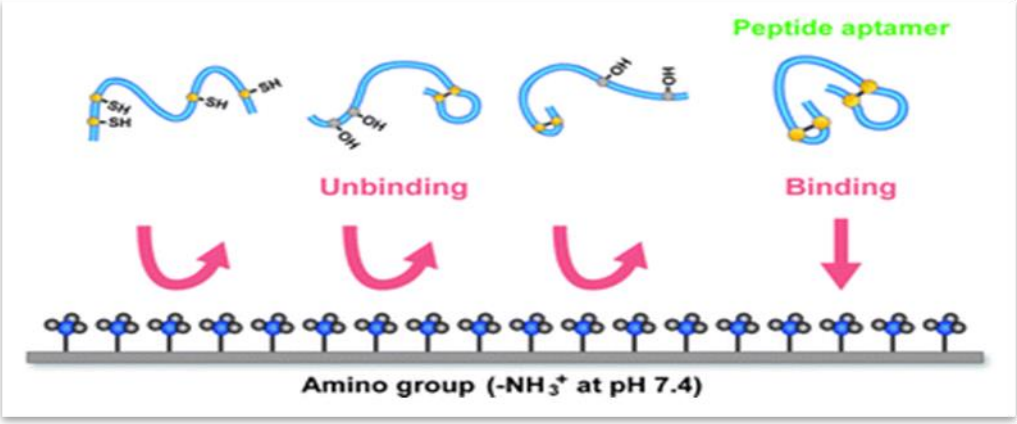

Figure 8: Amino group binding peptide aptamers with double disulphide-bridged loops.

Industry and Research Community: Commercial products and companies based on aptamers include the drug Macugen (pegaptanib) and the clinical diagnostic company SomaLogic. The International Society on Aptamers (INSOAP), a professional society for the aptamer research community, publishes a journal devoted to the topic, Aptamers. Apta-index is a current database cataloging and simplifying the ordering process for over 700 aptamers.

Conclusion: Aptamers are a promising new class of biomolecules with a wide range of potential applications in medicine and other fields. Aptamers are versatile, simple, stable, and modifiable, making them ideal for a variety of applications. Aptamers are a powerful new tool that has the potential to revolutionize the way we diagnose and treat diseases. They are versatile, robust, and easy to produce, making them ideal for a wide range of applications. One of the most exciting aspects of aptamers is their potential to be used in theranostics. By combining diagnostics and therapeutics into a single platform, theranostics could lead to more personalized and effective treatments for patients. For example, aptamers could be used to develop drugs that can target and deliver therapeutic payloads directly to cancer cells. This would minimize side effects and improve the efficacy of cancer treatment. Aptamers are also well-suited for use in point-of-care diagnostics. Because they are small and stable, aptamers can be incorporated into portable devices that can be used to diagnose diseases in the field. This would make it possible to diagnose and treat diseases in remote or underserved areas. Aptamers are already being used in clinical trials to treat a variety of diseases, and they have the potential to revolutionize the way we diagnose and treat diseases in the future.

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