

Pseudo aptamer expands aptamer's applications

Abstract

Aptamers are oligonucleotides, such as ribonucleic acid (RNA) and single-strand deoxyribonucleic acid (ssDNA) that can bind to almost all of molecules, small molecules and macro biological molecules, as their targets with high affinity and specificity due to their specific three-dimensional structures. Aptamer based oligonucleotides provide a novel molecule and an approach for the research and development of a new generation reagents in broad range of applications, particularly in biological and medical sciences, including diagnostic materials and therapeutic molecules. Conventional aptamers are constructed by nucleoside building blocks connected with diphosphate ester linker that is sensitive to nuclease and chemical hydrolysis, causing the degradation of the nucleotide chain. The solutions to this problem would be chemical modification either on the ribose sugar, or nucleobase, or phosphate linker. Almost all used aptamers are not net structures as original one; they were modified less or more. Unfortunately, none of these has completely resolved the problem without losing activities. Therefore, developing new structures is urgently needed. This review is mainly focused on the recent progress in the research and development from structure perspective, hoping to derive useful enlightenment and be able to guide and determine the choice of such projects.

Keywords: aptamer, aptbody, oligonucleotide, nucleobase, ribose, phosphate, pseudo-aptamer

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Abbreviation: BNA, bridge nucleic acid; DNA, deoxyribonucleic acid; LNA, locked nucleic acid; LDL-C, low density of lipoprotein c; PNA, peptide nucleic acid; RNA, ribonucleic acid; SAR, structure and activity relationship; THF, tetrahydrofuran

Introduction

Because of broad range of applications and distinctive properties of aptamer, the global market size was valued at USD 723.6 million in 2016 and is projected to grow at the compound annual growth rate (CAGR) of 28.2%,¹ and expected to reach \$8.91 Billion by 2025, growing rapidly. Aptamers and the derivatives are also referred to as "synthetic antibodies" or "chemical antibodies"²⁻⁵ that are able to bind with high affinity and specificity to almost all types of molecules as well as antigens, cells. Because of their unique properties, aptamers have a wide range of applications, particularly in biological and medical sciences, including diagnosis, therapies, forensics, and biodefense.⁶⁻⁹ So far, hundreds of aptamer reagents have been developed for the applications,¹⁰ which are faster, cheaper, and less or without the predictable problems associated with the production of recombinant antibodies. This review summarizes the recent technologies of modified analogous of aptamer, so called pseudo aptamers in this script.

Aptamer and their conjugates act in the manner of intermolecular interactions fundamentally, and as the ligands bind selectively to the targets via sturdier forces. The specific recognitions of these molecules are mainly attributed to their structures, expressed by the synchronous forces, such as h-bonding, charge-charge interaction, pi-stacking interaction, hydrophilicity or hydrophobicity, metallic chelating, and so forth. Therefore, multiple functionalities, as well as the resistances to nucleases and hydrolysis are critical in aptamer analogue design.

Aptamers are the single-strand structure of nucleotide chain, including DNA, RNA and the homologues. Comparing to small molecules, their remarkable properties are regulated by the sequence, the exclusive molecular weight, three-dimensional geometric shapes, the manipulative pairing, conformation, and density of charges, as well as rigidity etc. Once binding to the receptor, it will disable the function of the protein in the manner of proper conformed complex, or change the conformations of some proteins behaved molecular switches in some case.^{11,12} As recently developed, an important benefit of aptamer technology is that fine ligand allows for rapid and parallel identification of any actually targeted molecules simultaneously and automatically.¹³ Therefore, aptamer technology is well suited to address the growing needs for functional screen and validation of high-throughput bioactivities. To expand the applications of this technology, many studies have implemented, the modified aptamers inhibit the proteins not only in cell culture, but even more efficient in vivo models,¹⁴⁻¹⁶ which complements the gene knockout or siRNA methods because they provided highly valuable information at proteomics level. Recent technologies have been extended to the new structures of aptamer that are functionally equivalent at least to the conventional one.

Variety of structure changes makes aptamer technology more efficient and imposing, the applications have rapidly increased in many of the illustrated areas as outlined in Figure 1. Aptamers have been used as the new biomaterials along with increasing demands. The first drug approved by Food and Drug Administration (FDA) in 2004 is Macugen, a (polyethylene glycol) PEG conjugated aptamer against vascular endothelial growth factor (VEGF). It was used to treat age-related endovascular macular degeneration (AMD).¹⁷⁻¹⁹ It is an outstanding milestone of aptamer medicine.

It had been a while that aptamers have become well-known as an alternative antibody because these molecules overcome not only the weakness of biological antibody molecules but are superior also to the small molecules in selectivity. In terms of techniques, several types of *in vitro* screening processes have been used, including nitrocellulose membrane filtration, affinity gas chromatography, magnetic beads, and capillary electrophoresis-based selection. Various modified aptamers have also been used to diagnose disease and imaging.²⁰

Aptameric analytical techniques, such as electrochemistry, calorimetry, optics, mass-sensitive methods, etc., have already been developed for detections^{21,22} benefited by the advantages of the convenience of modification and the stability of the aptamer. In addition to medical and analytical applications, aptamers are also a promising material for use in many other fields, such as nanomaterials. Nevertheless, all these properties are the structure-based consequences, thus their applications can be subjected to enormous manipulative structure patterns. Since conventional structural aptamers developed and under developing had shown no destroying the surrounding cells, called 'magic bullets', including the biomarkers for the diagnosis of cancer metastasis, therapeutic drug delivery, and so forth.²³ Modified pseudo aptamers should be designed intentionally to conserve this property, particularly for cancer imaging and therapy.

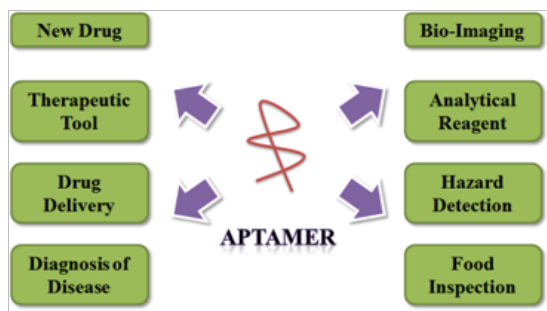


Figure 1 Aptamers for a variety of applications.

Conventional structure

The primary structure of aptamer is constructed as linear chain, determined by the sequences on the phosphodiester linker, on which the secondary structure habitually forms and then folds via complimentary rule. The rigidity of folded 3D structure is required for molecular interaction and recognition. This sequence-based aptamer can be prepared via the well-established solid phase synthetic approaches rapidly in large quantity by means of phosphoramidate chemistry.^{24–26} Conjugations can be easily done at the any steps in the solid phase synthesis, either on any building block or the termini.

The formed 3D structure should be relatively stable at the certain range of temperatures, especially at the room and biologic temperatures. The major interaction forces in the binding process are defined to be hydrogen bond and charge-charge interaction that are provided from native phosphodiester polymeric linker and the bases on loops.^{27–30} The concept of aptamer ligands binding to proteins was reported early 1980s for the study of human immunodeficiency virus (HIV) and adenovirus.^{31,32} In this investigation it had shown that the viruses have a high force of affinity and specificity for the encoding of small numbers of RNAs that bind to viruses or cellular proteins. A milestone of a substantial progress should be an *in vitro* selection program developed in 1990 as Figure 2, systematic evolution of ligands by exponential enrichment (SELEX)¹³ has now been a

basic technique to screen aptamers that direct to a variety of targets including small molecules or biomolecules.

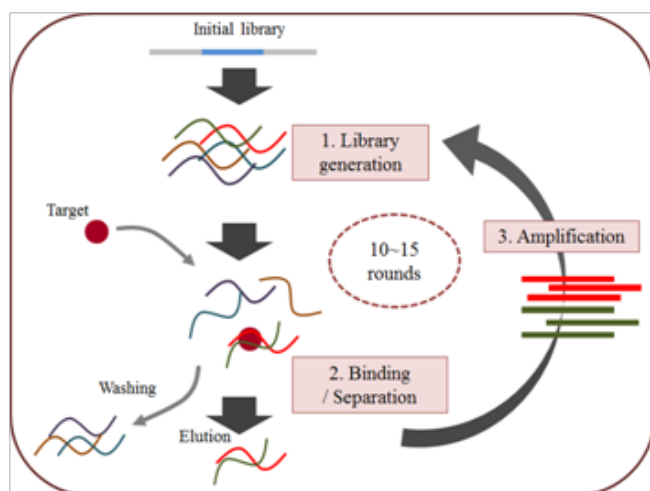


Figure 2 General SELEX.

Comparing with smaller molecules, even though many other ligands can bind to the same receptor, it has been observed, in many cases, that aptamers exhibit much higher binding affinity than those compounds that are derived from small molecule library, or natural product obtained by random screening,³³ suggesting that aptamer can be developed as new generation of medicines.

Comparing to random screen, the challenges in ligand design³⁴ are: first, the determination of the proper target and the insight of the complicated target, like biomarkers or cancer markers. The three-dimensional structure manipulation in design of ligand must impact the recognition and binding effectively. Second, the design depends on better understanding the interactions between the ligand and the receptor if not just random screening. Rational design, the molecular modeling, and computational approaches provide theoretic methods. Third, the drug delivery techniques towards the target should be considered, which mostly relies on the chemical and physical and biological properties of the molecule. Additionally, drug administration pathway, formulation and the stability of the ligand should also be considered in the design.

Further, considering antibody, the identification and production of monoclonal antibodies is a time-consuming and costive process in the screening of large number of clones. Clinical used antibodies inevitably are produced by large-scale cultivation of mammalian cells to meet the needs. In addition, immunoassays must also be used to confirm the activity of every new batch of antibodies, as the performance of the same antibodies will vary from batch to batch. In contrast, once aptamers are designed, they can be synthesized by chemical reaction rapidly with high accuracy and repeatability, which are more cost-effective than antibodies producing.³⁵

Additionally, the conventional constructed aptamers generally appear to have low immunogenicity and low toxicity, because nucleic acids are not usually recognized as foreign invaders by immune system. On the other hand, like an antibody, the derivatives of aptamer have significant immunogenicity and prevent subsequent administration.³⁶ However, antigens or immunogenic moiety conjugated with aptamer opens another door for artificial vaccine development.

Helix structure and self-folding are determined first by absolute Chirality of ribose and the flexibility of phosphodiester bonds. Once the aptamer sequence has been determined, the automated synthesizer can produce as much as needed, but the cost of chemically fabricating aptamer is equivalent to the cost of the antibody as the bio-product purchased, which may not be suitable for pseudo aptamer because of different synthetic methods. Aptagen LLC, for example, a global manufacturer for custom services, offers an ever-growing list of over 300 aptamers at prices of less than \$1-4 per microgram in just two days to two weeks, while the human monoclonal antibody takes 3 months to complete, and the average cost is about \$300/g.³⁷ The modifications of aptamer can be readily carried out to increase the desired biological properties, such as adding an inverted thymidine, PEG, amino, 2'-O-methyl, biotin, or fluorescent tag, and many other functional groups.³⁸

Pseudo aptamer from the linker changed backbone

As described above, the natural formed diphosphate ester backbone determines the special functionalities of nucleotides, its flexibility and the static charges help the entire molecule self-assembling, which should be preserved in any mimics.³⁹ However, any modifications on it may impact the functions less or more. Thus, rational design and modification should be helpful to decide the desired consequences. Several modifications and the utilizations are summarized below, among them, boranophosphates and thio- or dithio-phosphates upon the phosphoramidite are the most adopted modifications both in solution phase and solid phase synthesizing.⁴⁰

2'5' linkage

The adjacent bond switch from 3'-5' to 2'-5' occurs only on RNA ribose; the chemical copying of RNA templates generates product strands that contain 2'-5' connections as in Figure 3. When diphosphate ester linkage between 3'-5' hydroxyls switches to 2'-5', the diminished thermal and chemical stabilities of the duplexes reflect local structural changes, while the compensatory changes result in a global duplex structure with relatively minor effect. This intramolecular rearrangement can occur under certain circumstances at few positions chemically and biologically. It may cause heterogeneous constructions, and possibly homogeneous structure at every connection *via* chemical synthesizing. This rearrangement goes *via* the oxygen on the adjacent phosphorus to form a pent-cyclic intermediate that eventually breaks off as an oxyanion leaving group. In fact, many ribonucleases and ribozymes cleave RNA by an intramolecular phosphoester transfer reaction.⁴¹ In addition, few modifications on RNA single strand adjust their local structures to accommodate the perturbation caused by 2'-5' linkages between the two nucleosides, to ensure the neighbor nucleotides reducing the disruptive effects of the isomeric linkage and resulting in a minimal effect of global structure.⁴² Nevertheless, if the global structure of aptamer is homogeneously linked *via* 2'-5' phosphodiester, its functions and activities haven't been known yet.

Cyclic structure

The smallest cyclic dinucleotide has anti HIV activity, it is so called second messenger, targeting the protein that plays role as 'riboswitch', stimulates the innate immune response in mammals. There are also bacterial riboswitches that selectively recognize cyclic dinucleotides. It was reported that a natural riboswitch targeting 3', 3'-cGAMP is distinguished from the endogenous mammalian signal 2', 3'-cGAMP binding protein by its backbone connectivity^{43,44} as shown in Figure 4.

Sulfurization

The non-bridging oxygen atoms of the phosphodiester backbones of RNA and DNA aptamers can be substituted with one or two sulfur atoms, resulting thiolate -aptamers with phosphorothioate or phosphorodithioate linkages respectively. Such thioaptamers are known to increase binding affinity with their target, as well as enhance resistance to nuclease degradation.^{45,46} Unfortunately, if a modified aptamer possesses high proportions of phosphorothioate or phosphorodithioate linkages it appears to lose some specificity towards binding proteins than normal phosphodiester. Thus, the exact positions or sequences of the sulfur modified phosphodiester unites create extra questions that need to be addressed in design. The effect often attributes to both non-specific and specific interactions, since the recognition between nucleotide acid and proteins involves side chains and backbone interactions in the manner of the nucleic acid bases amino acid codes,^{47,48} hydrogen bonds,⁴⁹⁻⁵³ charge-charge interactions,⁵⁴⁻⁵⁹ hydrophobicity or hydrophilicity^{60,61} conformations,⁶²⁻⁶⁹ rigidity etc.⁷⁰⁻⁷²

Among the modifications on backbone, sulfurization is one of common ways. In doing so, dithiazol-one based derivatives as the most efficient sulfurization reagents have been employed to introduce sulfur element. The reaction can be carried out on the building blocks or at the any step of solid phase synthesis. If the sulfurization is implemented at the last step of solid phase synthesizing, it will create heterogeneous products with certain proportion,⁴⁵ or even homogenous products if excess amount of sulfurization reagent is employed, as shown in Figure 5.

Peptide nucleic acid

Peptide nucleic acid (PNA) as shown in Figure 6 is the synthesized polymer similar to DNA or RNA, it was designed as nucleotide mimic.^{73,74} Synthetic peptide nucleic oligomers have been used in molecular biology procedures, diagnostic assays, and antisense therapies in recent years. Due to their higher binding strength it is not necessary to design too long PNA oligomers to use, it usually requires normal oligonucleotide probes of 20-25 bases. The main concern of the length of the PNA-oligomers is to guarantee the specificity that is determined by secondary and self-assembled structures.⁷⁵ PNA oligomers in fact show greater specificity in binding to complementary DNAs, with a PNA/DNA base mismatch being more destabilizing than a similar mismatch in a DNA/DNA duplex. This type of binding strength and specificity applies also to PNA/RNA duplexes. All of these suggest that the changed backbone with similarities of geometric size, flexibility, and helix conformation properties are compatible to the pairing-able oligonucleotide.⁷⁶ One of the major advantages of PNA is hard to be recognized by either nucleases or proteases, making it resistant to degradation by enzymes. PNAs are also stable over a wide pH range and thermal conditions. Though unmodified PNA cannot readily cross cell membranes to enter the cytosol, covalently conjugating a cell penetrating peptide to a PNA can improve cytosolic delivery, poly glycine based PNA is an example as shown in Figure 6. PNA shows remarkably strong hybridization with complementary nucleotides, due to its non-charged backbone. However, low solubility and poor cellular uptake of PNAs limit their applications if no further modifications are employed.⁷⁷⁻⁷⁹

Together with PNAs, unlocked nucleic acids (UNAs) were developed and optimized for the applications. UNA monomers are acyclic derivatives of RNA lacking the C2'-C3'-bond of the ribose ring of RNA. UNAs have a highly flexible scaffold because

of removing the C2'–C3' bond,⁸⁰ however, nucleosides with UNA modification lead to the decrease of duplex thermal stability in both DNA and RNA.^{80–82} (Figure 7)

Boranophosphates

Boranophosphates are synthetic boronated nucleosides that have been studied as potential therapeutic and diagnostic agents.⁸³ The boranophosphate resembles the normal phosphate that the negative charge retains. However, the polarity of the molecule changes and the negative charge can be remains on the oxygen cation. Partitioning experiments have demonstrated that boranophosphates are more lipophilic than normal phosphates, this could allow for increased cellular uptake in delivery. Additionally, boranophosphates have increased nuclease resistance without affecting activity of RNase dissociation of RNA boranophosphate hybrids.⁸³

P-boronated oligonucleotides have been attracting much attention as potential therapeutic oligonucleotides.⁸³ In a study, researchers developed H-boranophosphonate oligonucleotide bearing a borano group and hydrogen atom on the internucleotidic phosphorus demonstrated that the novel P-boronated oligonucleotide is a versatile precursor to various P-boronated oligonucleotides such as boranophosphate, boranophosphorothioate, and boranophosphoramidate. The method was also applicable to the synthesis of a locked nucleic acid-modified boranophosphate oligonucleotide, which exhibited a dramatically enhanced affinity to complementary oligonucleotides,⁸⁴ as shown in Figure 8.

Phosphodiester unite modified by a borane tri-hydride (-BH_3) has behaved also of high chemical stability, high nuclease resistance, and potential of using in boron neutron-capture cancer therapy and increasing the activity of siRNA and so forth.⁸⁵

In a chemical synthesis, phosphodiester linker synthon or oligomer backbone can be treated directly by BH_3 -THF. Except for chemical methods, the enzymatic synthesis of BH_3 -ODNs has been achieved⁸³ with high optic selectively. As it is known that T7 RNA polymerase recognizes only the Rp diastereomer of nucleoside to introduce Sp-boranophosphate internucleotide linkages, so that Rp linkages cannot be incorporated into an oligonucleotide by the bio synthetic method.⁸⁶

Pseudo aptamer from nucleobases modified structures

Nucleobases are major nucleotide moieties that determine aptamer not only to fold properly via complementary rule, but also to stabilize the 3D conformations, in contrast to proteins that are readily denatured and lose their intrinsic tertiary structures at higher temperature. Aptamer has good thermal stability and can maintain a repetitive cycle between the structural denaturation and refolding. The biggest advantages of oligonucleotide-based aptamers over protein antibodies are its simple structure and thermal stability. Furthermore, the aptamer can restore the native conformation and can bind to the target receptor over again after re-annealing, on the contrary, the antibody denature is irreversibly once occurred. Therefore, aptamers can be used in a broad of assay conditions.⁸⁷

The bases modifications can be very diverse. It had been reported that the introduction of unnatural nucleosides is sufficient to support the replication of an unnatural base pair on a high copy plasmid. It was demonstrated that *E. coli* infected by unnatural bases is also efficient for bio molecular synthetic applications. The examples of the base's changes are shown in Figure 9. However, their pairing capability on oligomer hasn't been known well yet.^{88, 89}

It is necessary to better understand the structures and the properties relationships of modified bases. Any types modifications represent passive mark, even the addition of small groups, such as methyl, affects the ability of bases to pair, to stack against neighboring bases, to adopt certain conformation, to fold favorable structure, and to interact with proteins consequently. Base modifications arise from chemical substitutions more work is needed to address how such changes influence base pairing, helix stability, folding and conformation.^{90,91}

It should not be missed obviously that the conjugation on the amine at the desired positions has been one efficient way of modifications via the simple chemical reactions, such as N-alkylation, N-coupling. These simple bases modifications can promote the pseudo aptamer properties, such as the conformations and global structures. Because that the primary amines on cytosine, guanine, adenine are readily to hook up with numbers of groups through nucleophilic substitutions on the desired building blocks that will be inserted into the sequence at any wanted steps, modifications can be one or more on the scaffold.

Other modifications on bases thymine and uracil can be the halogenated, 2-fluoro-modified pyrimidines, substituted amines and 2-OH purines, and many other functional groups of labeling moieties like fluorophores and quenchers for example, which greatly facilitate the identification and search of biosensors or discovering diagnostic materials.^{92–93} Overall effects of bases' modifications should attribute also to the loop regulation and complementary pairing and conformational change.^{94–99}

Pseudo aptamer from ribose modified construction

As mentioned above, pseudo aptamer is a type of artificial oligonucleotides, thus, modifications of single strand of oligonucleotide sugar rings have also been extensively investigated to improve the binding ability and the resistance to nucleases.¹⁰⁰ UNAs is the one of this type of modifications as mentioned in Figure 7. The ribose building blocks are so essential because its unique natural absolute Chirality determines the entire molecular global configurations, and benefits the safety insuring because its metabolic process creates nontoxic segments.^{101–104} However, it's considerable that any changes on ribose may create unsafe problem.

Bridge nucleic acid (BNA) or Locked nucleic acid (LNA):

Chemical modification directly on the ribose is not so easy, since core structure has less position or functional groups for reactions, except the 2-hydroxyl. Among the modifications, locked 2',4' hydroxyls nucleic acid (LNA) is developed, however, this modification is limited on RNA building block, which might not affect the activity of its constructed aptamer. Although most 2'-linked sugars were in the expected 2'-endo conformation, some were partially or fully in the 3'-endo conformation, suggesting that the energy difference between these conformations was relatively small,¹⁰⁵ as shown in Figure 10.

Bridged nucleotides (BNA) can be incorporated into DNA or RNA oligonucleotides at any desired sequence or position. These monomers are synthesized individually as special building blocks. The conformation of bridged ribose enhances base stacking and pre-organizing the backbone of the oligonucleotide, significantly increases their hybridization properties, it may because that high rigidity causes helix formation. The incorporation of BNAs into oligonucleotides allows the production of modified synthetic oligonucleotides with higher binding affinity against DNA or RNA, better selectivity, stronger and more sequence selective triplex-forming characters, pronounced hi-

gher nuclease resistance than Sp-phosphorothioate analogues, as well as better water solubility.¹⁰⁶⁻¹⁰⁸

A new type of BNA analogs were designed by taking the length of the bridged bonds into account. A six-membered bridged structure with a unique structural feature (N-O bond) in the sugar moiety was designed to insert a nitrogen atom. Nitrogen atom improves the formation of duplexes and triplexes by lowering the repulsion between the negatively charged backbones of phosphates. These modifications allow controlling the affinity towards complementary strands, regulating resistance to nuclease. The properties of these analogs were investigated and compared to those of previous 2', 4'-BNA (LNA) modified oligonucleotides. 2', 4'-BNA-NC modified oligonucleotides with these profiles show great promise for applications in antisense

and anti-gene technologies.¹⁰⁸

BNA-based antisense therapeutics inhibits hepatic PCSK9 expression, resulting in a strong reduction of the serum LDL-C levels of mice. The findings supported the hypothesis that PCSK9 is a potential therapeutic target for hypercholesterolemia. The researchers announced that BNA-based antisense oligonucleotides induced cholesterol-lowering action in hypercholesterolemic mice. The same group reported that the 2', 4'-BNA-[NMe] analog, when used in antisense oligonucleotides, showed significantly stronger inhibitory activities which is more pronounced in shorter (13-16mer) oligonucleotides. Their data conclude that the 2', 4'-BNA-[NMe] analog may be a better alternative over conventional LNAs.¹⁰⁷

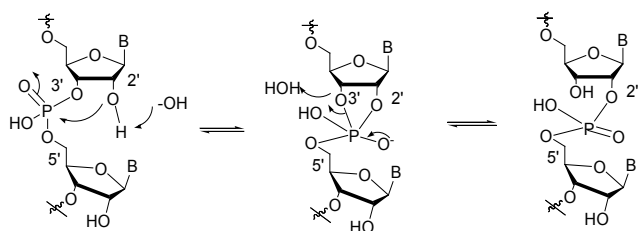


Figure 3 3'-2' rearrangement.

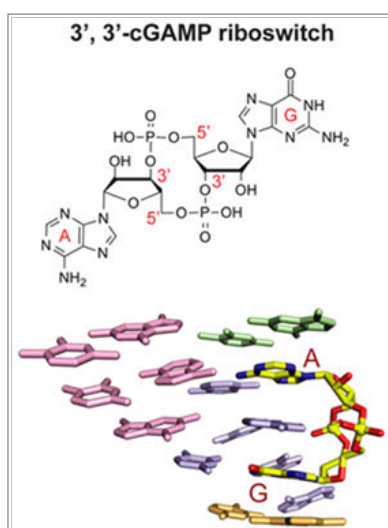


Figure 4 Dicyclic Structure.

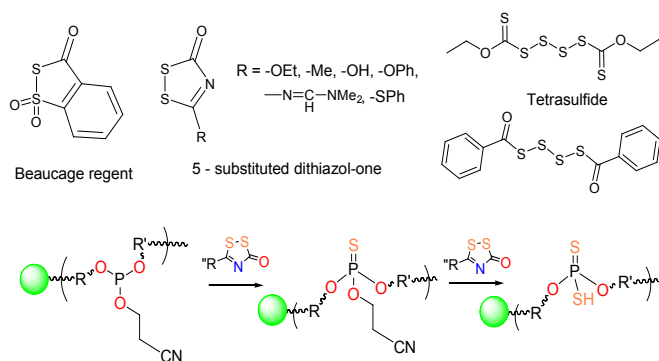


Figure 5 Common Sulfurization Reagents.

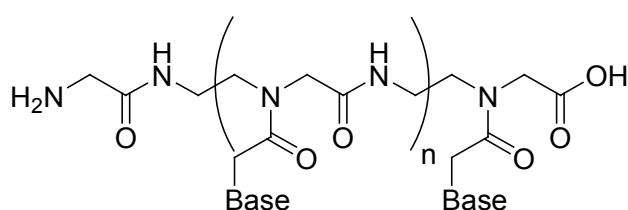


Figure 6 PNA Structure.

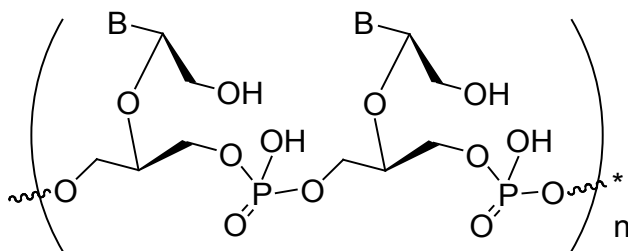


Figure 7 UNAs Structure.

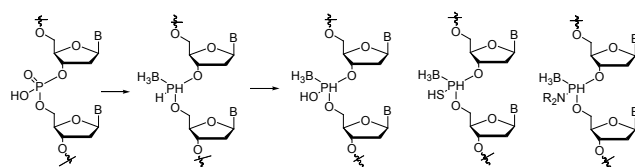


Figure 8 P-boronated Structures.

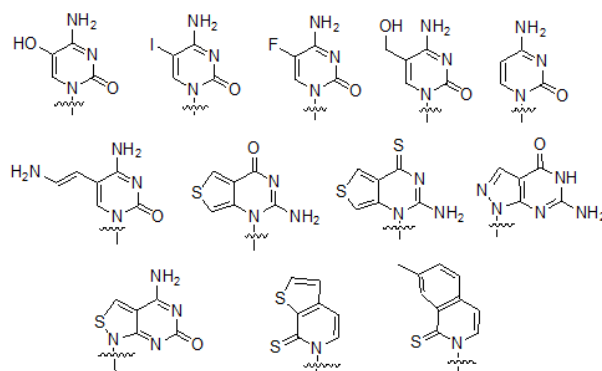


Figure 9 Modified Bases.

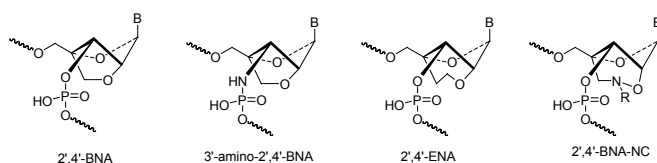


Figure 10 BNA or LNA Structures.

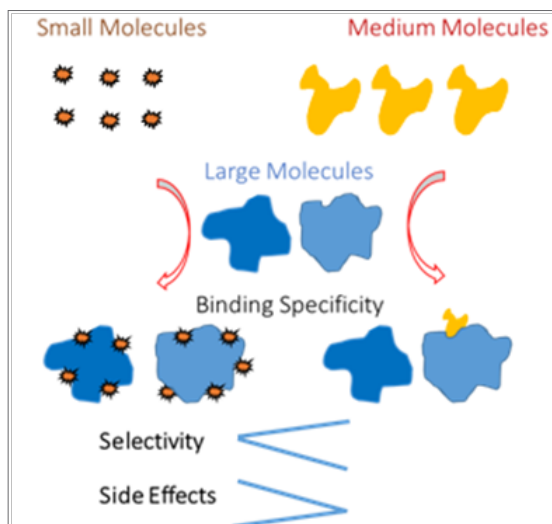


Figure 11 Intermolecular Interaction.

Discussion

Pseudo aptamer as an artificial nucleotide mentioned above have been developed to mimic natural nucleotide, it has expanded to more broad applications. These molecules are also called 'Aptbody' or 'artificial antibody' or 'chemical antibody'. We assume that about 30Å(angstrom) length or six bases unites at least are necessary for folding and loop formation in a single strand oligonucleotide. The minim number of building units should be more than six to ensure the stem and loop constructed simultaneously.

All of the information together, it is not difficult to understand that the main challenges and factors in pseudo aptamer design as below will impact applications of the molecules eventually, and these affects and factors would also crossly affect each other:

- Appropriate linkage backbone affects stability, helix formation.
- Appropriate length determines the minimal size and folding.
- Appropriate flexibility affects secondary structure and conformation;
- Appropriate helix affects nucleotide hybridization, and target binding.
- Appropriate hydrophilicity affects solubility, cell membrane transportation.
- Appropriate static charge affects interaction, binding, targeting, and delivery.

Overall, one of the major limitations of conventional aptamer is rapidly clearance from blood circulation due to nuclease degradation. However, this limitation can be effectively resolved by chemical modification, in the manner of pseudo aptamer or aptbody, which includes conjugations as well. In practice, polyethylene glycol, or a lipid, or cholesterol bonding can increase the retention time in blood stream and cell membrane penetration. Modified nucleotides contain

denatured bases, sugars, and nucleotide-linked groups that can be used as the pseudo building blocks in the constructions of pseudo aptamers to improve nuclease-resistant, chemical, physic, and biological properties. It has to be pointed out, due to the high chargeability of nucleotide-based compounds can result poor cell-penetration ability, lower bioavailability, even drug delivering difficulty, conversely, it would be effectively improved also by chemical modification.

Conclusion

Upon the numerous researches and developments, further modifications and structure optimizations appear to be more essential. Outstanding challenges in oligonucleotide medicine development would be optimization of chemical architectures to ensure enable robust clinical activity and long-term safety. A hypothetic thought comes out upon the conventional structure and activity relationship (SAR) of aptamers, either the core structure or the conjugated moiety can conduct to the multiple catalytic domains as recognition or binding sites on the complex receptor simultaneously, which may at least induce free energy reducing in the binding process, and the ligand-receptor recognition more accuracy. Therefore, the modified pseudo ligand could be better compatible to target and affect the biological function of the protein. Whether the conjugate may or may not be able to release therapeutic or cytotoxic portions after binding to the site, the multiple interactions of a pseudo aptamer itself will highly promise to affect the protein activity consequently, illustrating the medium geometric size would be helpful for specific recognition, like the relationship between a key and a lock. It has been reported that the dissociation constants and the binding ranges of pseudo aptamers for various targets are from micromolar to nanomolar, and picomolar in some cases.¹⁰⁹

In comparing to small molecule drugs, a medium size molecule has higher specificity that reduces the chances of adverse side effects and complications; both are often found in the use of traditional drugs of small molecules. It was reported that one aptamer binds to theophylline exhibiting greater than 10,000-fold affinity than caffeine binding, whereas theophylline and caffeine differ in structure just one single methyl group.¹⁰⁹ Figure 11 tries to illustrate these interactions.

In comparing to the large biomolecules, on the other hand, the size of the pseudo aptamer is only a small proportion, and therefore it has greater capacity to enter the cell cavity, and bind to the particular target that is highly compatible in between. As for traditional antibodies, it has the problem of cross-reacting or "false-positives". The efficiencies of pseudo aptamer ligands however, would be evaluated through *in vitro* and *in vivo* screening on various cell lines and tissues, the more effective candidates would be obtained in further development.

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Conflict of interest

Author declares that there is no conflict of interest.

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