SYSTEMATIC REVIEW

NON-SELEX-BASED IN-SILICO MODELED APTAMERS AGAINST SARS-COV-2 PROTEINS: A SYSTEMATIC REVIEW

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ABSTRACT

Background:

The SARS-CoV-2 transmitted COVID-19 pandemics have constituted a worldwide health challenge. Effective diagnosis and treatment of COVID-19 demands that a more reliable and rapid detection protocol should be adopted to replace the real time Polymerase Chain Reaction (RT-PCR) currently in use. Several researches were focused on the possible application of Aptamers and aptamer-based biosensors for swift point-of-care detection of the Covid-19 virus. Target-specific aptamers are generally developed through selection protocol referred to as Systematic Evolution of Ligands by Exponential Enrichment (SELEX). Conventionally, computational tools are used for post SELEX analysis, optimization and validation of SELEX-developed aptamers. SELEX procedure is however tedious, prohibitive, and time-ineffective.

Objective:

The aim of this review is to identify studies that present new aptamers modeled *in silico* against target proteins in SARS-CoV-2, high lightening the particular *in silico* methods employed

Methods:

Relevant articles that employed *in silico* approaches in designing Aptamers against SARS-CoV-2 proteins, retrieved from Scopus and PUBMED databases, screened and analyzed.

Results:

The study revealed that *in silico* design of aptamer typical entails construction of Aptamer library, target selection, structural modeling of apatmer, molecular docking, optimization and molecular dynamics (MD) simulation.

Conclusions:

In silico design of Aptamer is therefore a promising time- and cost-effective replacement to SELEX protocol.

Keywords: Aptamer, COVID-19, SARS-CoV-2, In-silico, SELEX

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as named the Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses, has distressed families and disturbed healthcare systems across the globe [1] . Detecting SARS-CoV-2 infections early is paramount in the control of COVID-19 pandemic [2]–[4]

The COVID-19 is currently being detected through either a serological and diagnostic tests protocol [3]. All the tests that examine patients for antibodies raised against SARS-CoV-2 are grouped as Serological tests. Such test aim to know individuals in a population that may still be at risk of COVID-19, whereas, the generally approved test now, being reverse transcription-polymerase chain reaction (RT-PCR) [5] targets and detect specific viral antigens, alongside other nucleic acid amplification tests (NAATs) like transcription-mediated amplification (TMA) and loop-mediated isothermal amplification (LAMP) are grouped as diagnostic tests [4]. NAAT-based molecular tests are expensive and still require to be performed in a laboratory or clinic. Developing an alternative point-of-care (POC) detection measure is therefore critical and urgent.

Aptamer technology aimed at developing oligonucleotides that bind specifically to a wide range of analytes (molecules, antigens, whole prokaryotes or whole cell of eukaryotes) has emerged in recent years as molecular recognition measure with great potentials in the diagnosis and therapy of several diseases [6] including COVID-19 [7]. SARS-CoV-2 possesses four (4) key structural proteins [8], a spike (S) glycoprotein, matrix (M), the nucleocapsid (N) proteins, the small envelope (E), and several other accessory proteins. N is located inside the virion whereas S, M, and E are attached to the surface envelope. The structure of the virion, figure 1, also reveal a spherical protein–lipid shell [3] apart from the nucleocapsid, The S protein is a fusion glycoprotein that plays vital role in the virus' interaction and binding and its entry into the cell. It experiences proteolysis to yield S1 and S2 subunits. The receptor-binding domain (RBD) that interacts with the receptor angiotensin-converting enzyme II (ACE2) of the host cells is born on the S1 subunit. Whereas the binding is facilitated by the S2 subunit [9]. Several researches therefore target the S protein for COVID-19 vaccines development as well as for diagnosis and therapy [2].

Target-specific aptamers are generally developed through selection protocol called Systematic Evolution of Ligands by Exponential Enrichment (SELEX). SELEX procedure often begins with a random ssDNA or ssRNA library and proceeds via the iteration of a target-driven PCR amplification phase. As the iteration progresses, the amplification products are being enriched with a pool of oligonucleotides demonstrating high specificity to the target. Aptamer sequences resulting from SELEX process are conventionally being analyzed via next-generation sequencing (NGS), thereafter, *in silico* optimization and validation studies could be carried out with the corresponding computational tools [4] as in Figure 2.

Conventionally, post SELEX computational analysis is being used to optimize and validate SELEX-designed aptamers. SELEX procedure is however due to the tedious, prohibitive, time- and cost-ineffective. *In silico* tools provide researcher different molecular modeling methods for aptamers design resulting in novel aptamers having improved target specificity. Figure 3 presents a typical workflow in aptamer

modeling against specific targets [10]. The basic operations includes; prediction and optimization of Aptamer structure (secondary and tertiary), docking of aptamer against the target wherein the Aptamer/target complexes having the lowest binding energies are carefully chosen to evaluate the stability of the complex by molecular dynamic simulations. The interactions between the Aptamer and target can then be analyzed and improved via chemical modification or mutation.

In this review, we summarize the few studies that present new aptamers modeled in silico against target proteins in SARS-CoV-2. The review can give insight as well as direction to researchers to employ and optimize the methods towards improvement and Aptamer performance and possible application. The difference in the nature of Aptamer (ssDNA/RNA/peptide) being modeled and the source of the starting materials is the uniqueness of our review.

METHODS

Document Search and selection criteria

The search for studies on in silico approaches in designing Aptamers against SARS-CoV-2 proteins was achieved by searching the keywords, titles, and abstracts field of Scopus and PUBMED databases. Studies that utilize SELEX protocol in developing Aptamer against SARS-CoV-2, and those that stemmed from a previous SELEX experiment were excluded. Only original research articles were included. Our search and post-search analysis followed the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) [16] statement. All available data obtained from the two databases reporting a non-SELEX in silico approaches in designing Aptamers against SARS-CoV-2 proteins were included. We read through titles and abstracts to screen the search result. We also screen by reading the entire articles in the second screening stage. Figure 4 presents the flowchart of the study selection.

Research strategy

The last document search was done on February 28, 2022. The inclusion criteria were as follows: keyword identification, synonyms based on relevant studies, and the use of boolean operators: "OR","AND" and " * ". We carried the search using the following components on both databases: ("in silico" OR comput* OR "de novo") AND (SARS OR covid) AND Aptamer.

RESULTS AND DISCUSSION

Study selection

We found a total of 46 studies on the Scopus database whereas on PubMed we found 35, summing up to 81 studies. We excluded 33 duplicate studies from the databases, thereby remaining 48 studies. Study inclusion and exclusion criteria resulted in four (4) studies selected for this review. In order to discuss the data from the four articles appropriately, other thirteen (13) studies that added information on coronavirus study or/and Aptamer development found outside the search performed were also included, totaling 17 articles included in this review.

In silico Aptamer Design

Objectives

In silico Aptamer design is a strategy that involves the usage of computer-based simulations to model, optimize and validate aptamer-target interactions. Conventionally, Aptamers molecular design is carried out computationally (either by screening existing

library or by de novo design), the sequences are subjected to molecular docking against the 3D model of target protein (for protein targets) to examine the strength of binding of each of the Aptamer sequences with the target. The best performing sequence is therefore optimized in silico to improve its binding potentials with the target, studying the Aptamer/target complex interaction by molecular dynamics simulation. The procedure is termed Structure and Interaction Based Drug Design (SIBDD) as in figure 5.

De novo Design protocol (Non-SELEX)

Two general methods have been employed in in silico construction of Aptamer. One approach involve the optimization of previously SELEX-identified aptamers whereas, the second excludes the time and cost ineffective SELEX protocol. We have solely focused on the latter in this review.

We summarized the design of two (2) ssDNA-one (1) RNA- and one (1) peptide Aptamer, as in table 1, that have so far been design against SARS-CoV-2, exclusively via in silico methods.

Mironov et al. [11] presented and applied a de novo method of creating a unique ssDNA library without template Aptamer. A ssDNA aptamer having high affinity for the SARS-CoV-2 spike protein' RBD domain was designed de novo. They modeled the DNA aptamer by choosing an hairpin initial shape (because of docking compatibility with the concave upper region of the RBD protein). The design was made to yield a 16mer sequence having a central variable "NNNN" loop of four nucleotides and two continuous self-complementary regions at the 5' and 3' ends (5'-GGAATTNNNNAATTCC-3'). All the possible nucleotide combination variability was considered for each region resulting in an arrangement of initial aptamer library having 256 entries.

A potential RNA aptamer was design in silico against a significant but less studied NSP10 (non-structural protein of SARS-CoV-2). The Shuffle seq program of EMBOSS package was used to randomly generate several RNA sequences, which were screened by some predetermined criteria viz: > 40% of GC content, RNA secondary structure having minimum free energy less than -5.7 kcalmol-1 and last three of 5' and 3' end nucleotides are complementary with each other [8]. The screened Aptamer were therefore selected for the downstream in silico research.

The capability of G-rich sequences to form four-stranded structures was employed by Gupta et al. [6] to design G-quadruplex DNA aptamers. The unique structure improved the Aptamer's binding affinity to the target. One hundred (100) DNA sequences forming a pool of a G-quadruplex Aptamer were randomly collected from the QGRS database. Several computational mutations strategies including duplication, four-base pieces' translocation, truncation and loop translocation were carried out on the aptamers to develop the library of 10,500 sequences of ssDNA.

Peptide aptamers, with amino acid ranging from 5 to 20 residues are being designed with binding affinity for their targets at specific. The design of peptide Aptamers against a target molecule, in an infectious agent begins with the insertion of an appropriate peptide into a scaffold protein. The peptide is often generated from the host, at the region of the attack by the infectious agent [13]. Scaffold proteins are mostly monomers, stable and rigid protein core that manifests flexible interaction target surfaces. Selecting the proper scaffold first step

in creating a library peptide aptamer for further analysis and studies. A novel peptide aptamer was designed by Devi & Chaitanya [12], using bacterial Thioredoxin A as the scaffold protein and peptide having 18 residue (N-AKTFLDKFNHEAEDLFYQ-C) which was inserted between CGPC motifs of the scaffold protein. The inserted peptide was selected from ACE2 region of human which is the target of the RBD of SARS-CoV-2 at the outset of the infection process. In silico evaluation of the performance, then, optimization and analysis of the designed peptide Aptamer followed.

Structural Modeling

Structural prediction and modeling of designed aptamer evaluates and prepares them for docking. The structural elements and arrangement depicts the stability of the Aptamer and hence the possible performance on docking analysis [14], [15].

Kothandan et al. [8] screened 10 RNA aptamers for docking study. The 2D structures of the RNA Aptamers of were prediction by RNA Fold of Vienna RNA software suite whereas the 3D structural prediction was done with RNA Composer. All the RNA aptamers were found to form secondary hairpin structure having at least 5 base-to-base pairing. Hairpin structures generally enhances stability for docking process.

Gupta et al. [6] used the online Mfold server to predict the 2D structures of reformed ssDNA aptamers whereas their 3D structures were predicted using Rosetta server. Modified aptamers structure had a sequential arrangement of hairpin, loop(s) and stem(s) which are characteristic of high specific binding affinity of aptamers.

The 2D structure of the peptide Aptamers from the library created by Devi and Chaitanya [12] were predicted with PSIPRED and RaptorX Property. But their 3D structural modeling was performed using Iterative Threading ASSEmbly Refinement (I-TASSER) Server and validation was done with SAVES v5.0 server. ERRAT score provided by the SAVES server showed the analysis of non-bonded atom–atom interactions in comparison with reliable high resolution crystallography structures. The server also provided a visualized dihedral angles psi (ψ) and phi (ϕ) of amino acids by a Ramachandran plot. GalaxyRefine server was used to refine the modeled 3D structures of the Aptamer. The validation results showed that 88.3% , 9.9% , 0.9% residues were predicted to be in the most favored, additional allowed and allowed regions respectively whereas only 0.9% residues were predicted to occur in the disallowed region. ERRAT score predicted 93.2773% over quality of the modeled peptide Aptamer, making it suitable for the docking studies and other downstream analysis.

Molecular Docking and MD Simulation

The aptamer-target binding potentials and stability of binding in the studies under review was evaluated by molecular dynamics using GROningen MAchine for Chemical Simulations (GROMACS). For every Aptamer modeled by Mironov et al. [11], they predicted ten binding sites. The molecule variant that was predicted to have the most binding sites located in the part presumed to be responsible for the recognition of the ACE2 protein by the virus was selected for optimization to improve its

potentials to bind with the target. The MD structures of RBD/aptamer complexes of Mironov et al. [11], were optimized at the semi-empirical density functional-based tight-binding (DFTB). Several amino acid replacement processes were carried out, to achieve a new Aptamer with better binding profiles. Interaction energies of the optimized structures were derived using the fragment molecular orbital (FMO) approach.

The screened RNA aptermers in the study of Kothandan et al. [8] were docked to NSP10 using HADDOCK v2.2. The activities of two other non-structural proteins, NSP16 and NSP14, depends on NSP10 as it stabilizes the methyl. Both NSP16 and NSP14 methyltransferase enzymes share some common sites of interaction with NSP10, however the stability of the virus depends on the interaction of NSP10 with NSP14. The binding affinity of the RNA Aptamer with NSP10 was studied by the docking analysis and optimization protocol thereby inhibiting the formation of NSP10-methyltransferase complex. The conformational stability of the aptamer-NSP10 complexes was analyzed in comparism with the apo-protein by plotting RMSD. The comparative dynamics study revealed Lys43, His80, Lys93, Tyr96 as the specific residues at which the RNA aptamers bind stronger with NSP10. The RNA-based aptamers were therefore proposed for synthesis and experimental development as potential anti-viral agents against SARS-CoV-2 after numerous clinical studies.

The G-quadruplex Aptamers of Gupta et al. [6] were designed against SARS-CoV-2 spike protein. Molecular docking studies was performed using HADDOCK online web server. The molecular interaction resulted in ten aptamers having high affinity score which were used for subsequent analysis. PLIP online web server was used to determine the Aptamer-target interaction sites for each Aptamer. From the results it was observed that all aptamers almost had interactions with amino acids in RBD, NTD, and HR1 domains.

The molecular docking analysis was performed with ClusPro 2.0 web server to evaluate the binding interaction of the peptide aptamer/SARS-CoV-2 S-protein RBD complex whereas the stability of the complex was evaluate and analyzed using GROMACS program. The binding of the peptide Aptamer to the RBD of SARS-CoV-2 S-protein was found to be comparative with the binding of the human ACE2 to the viral target, making the peptide Aptamer a potential competitive inhibitor of the virus. The evaluated energy parameters and stability index predicts the peptide aptamer to be soluble, nonhemolytic and anti-inflammatory.

Limitations of in silico Design

When aptamers are designed in silico, no matter the level of its performance efficiency by theoretical evaluation, an in vitro experimental evaluation or/validation is imperative [4], [10], [11]

CONCLUSIONS

The huge number of SARS-CoV-2-infected persons, and much more being at risk of SARS-CoV-2, demands easy, accurate, fast, and low-cost diagnostic technologies. None-SELEX in silico Aptamer design therefore is a potential approach to generating highly specific

detection and inhibitions agents against SARS-CoV-2 as well as other infections and ailments.

In silico methods in the design and analysis of Aptamer properties, structural prediction, target-binding analysis and evaluation, and optimization has proved effective for several target molecules. The studies reviewed in this article have also corroborated the potentials of in silico tools in Aptamer-based research. Although, experimental validation is required for aptamers designed computationally, however the in silico approach provides a platform to manipulate and control the binding affinity and properties of the Aptamer. Improving computational skills in sequence manipulation and optimization may therefore provide better performing Aptamers in subsequent *in silico* based design.

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Table 1: Summary of Reviewed *In silico*-deigned Aptamers

Type of Aptamer	Target	Source of Aptamer	In silico Methods	Reference
ssDNA	S protein	De novo synthesis	2D and 3D structural prediction, docking, MD simulation	[11]
RNA	N protein	Random generation	2D and 3D structure prediction, docking, MD simulation	[8]
ssDNA	S protein	Random generation	2D and 3D structure prediction, docking,	[6]
peptide	S protein	scaffold protein + ACE2-based peptide	2D and 3D structural modeling, docking, MD simulation	[12]

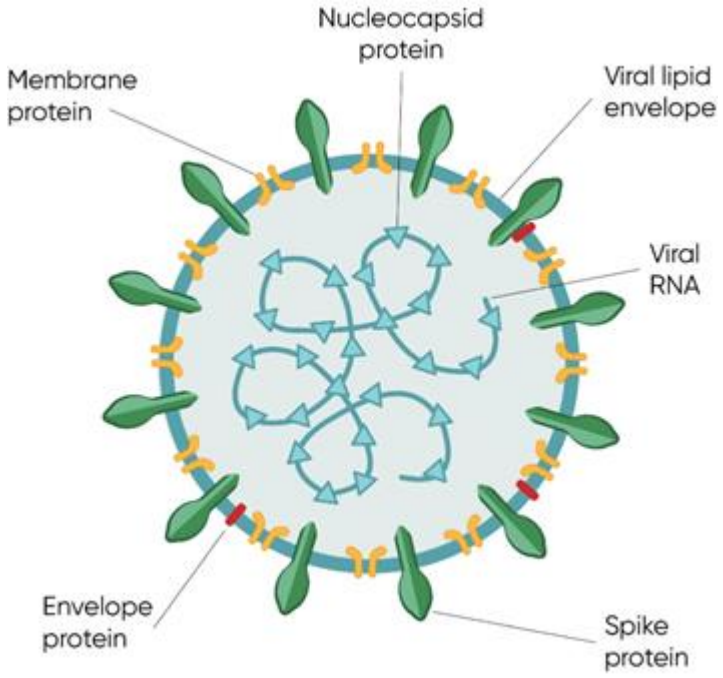

Figure 1: Structural proteins in the SARS-CoV-2 virion

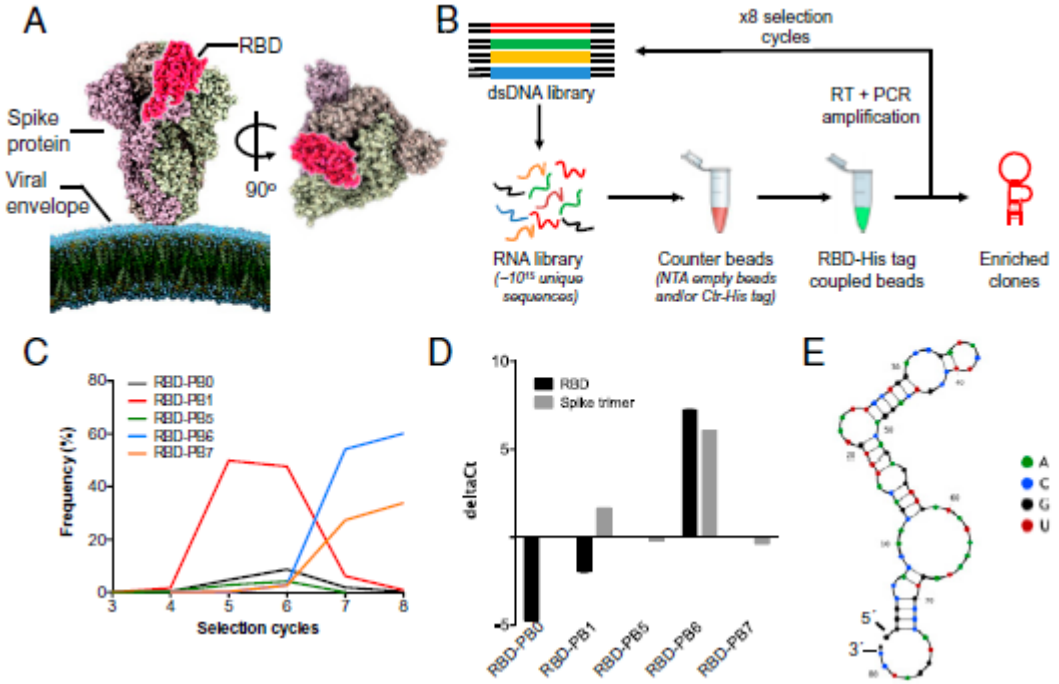

Figure 2: An RNA aptamer selection against RBD. [A] Spike protein top and side view [B] Selection scheme by SELEX [C] New Generation Sequencing data highlighting frequency (%) of the most typical RNA clones from the SELEX process. (D) The binding assay (qPCR) of five selected clones to RBD [E] RBD-PB6 secondary structure

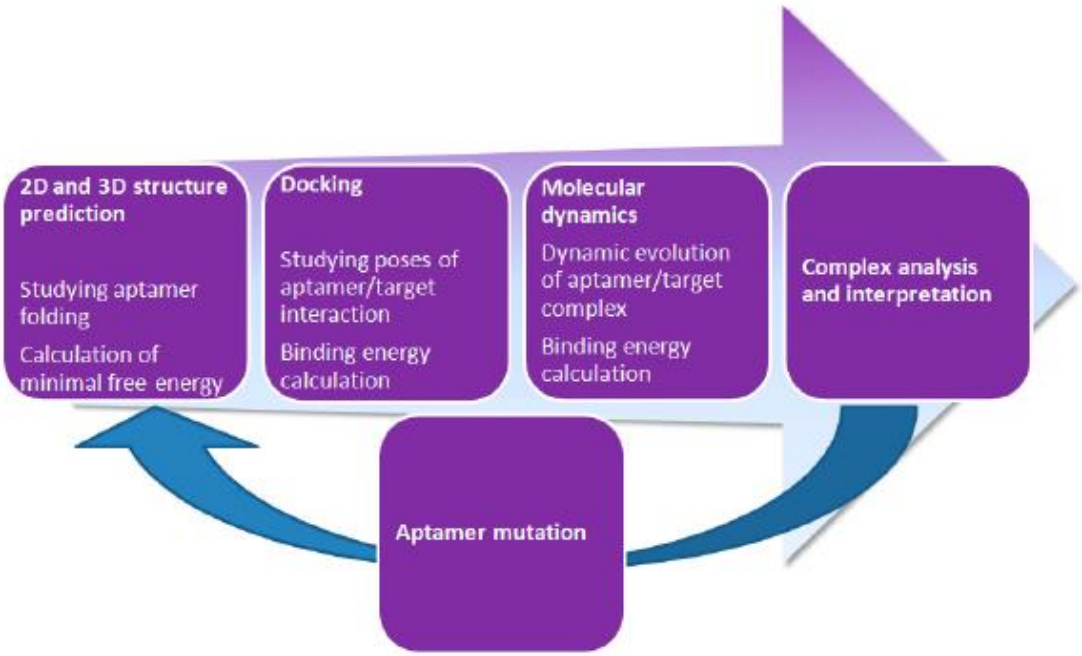

Figure 3: In silico methods in aptamer design: A typical workflow

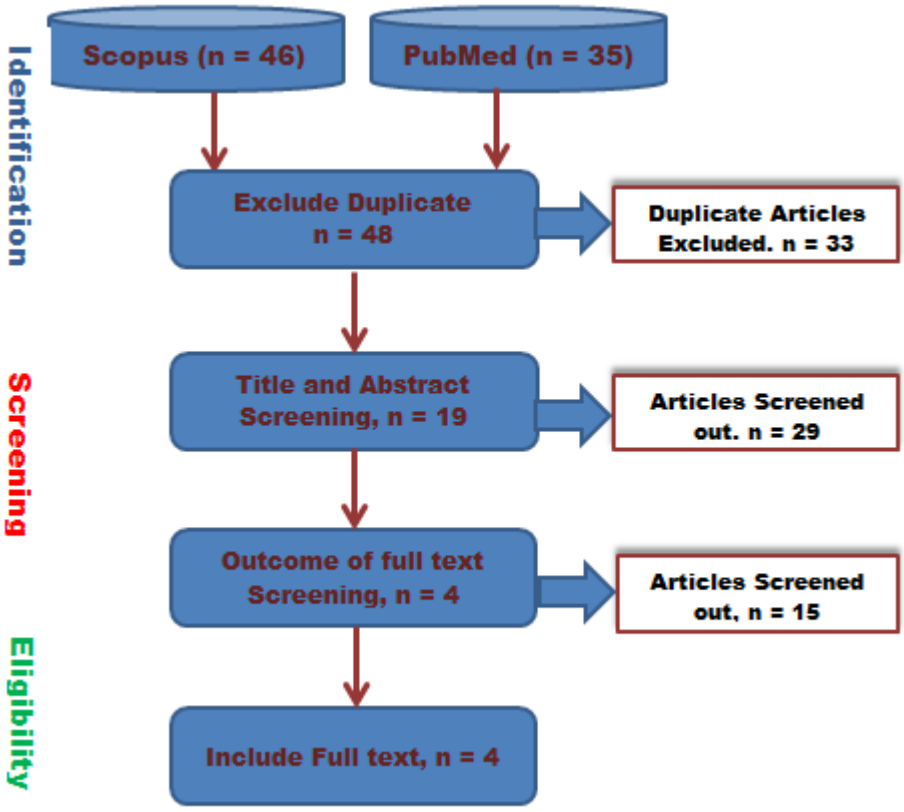

Figure 4: Flow Chart of Selection Process

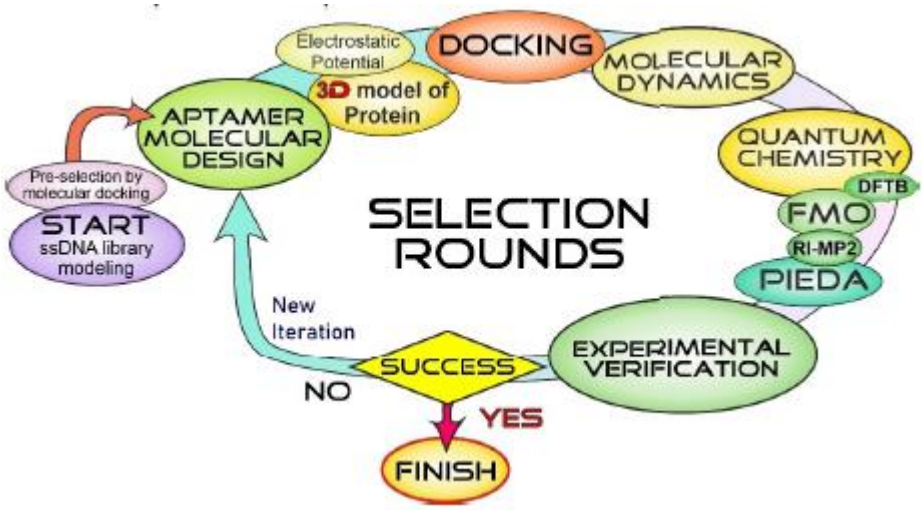

Figure 5: Steps Structure and Interaction Based Drug Design (SIBDD). Including: molecular design of aptamer; modeling 3D structure of the target protein; molecular docking of aptamers to the target; molecular dynamics simulations of the aptamer/target complexes; mechanical quantum analysis of nucleotide-residue interactions using DFTB, FMO, RI-MP2, or PIEDA; then finally experimental verification of the binding.