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Review

Aptamers targeting SARS-CoV-2: a promising tool to fight against COVID-19

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SARS-CoV-2, the causative agent of COVID-19, remains among the main causes of global mortality. Although antigen/antibody-based immunoassays and neutralizing antibodies targeting SARS-CoV-2 have been successfully developed over the past 2 years, they are often inefficient and unreliable for emerging SARS-CoV-2 variants. Novel approaches against SARS-CoV-2 and its variants are therefore urgently needed. Aptamers have been developed for the detection and inhibition of several different viruses such as HIV, influenza viruses, Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV. Aptamers targeting SARS-CoV-2 represent a promising tool in the fight against COVID-19, which is of paramount importance for the current and any future pandemics. This review presents recent advances and future trends in the development of aptamer-based approaches for SARS-CoV-2 diagnosis and treatment.

Highlights

Aptamers have been successfully used for the diagnosis and therapy of a broad spectrum of pathogens including bacteria, parasites, and viruses.

The wide application of aptamer-powered technologies in viral disease suggests that aptamer-based methods hold promise in combating SARS-CoV-2.

Aptamer-based technologies have been recently developed for both the detection and treatment of SARS-CoV-2 infection.

Aptamers targeting SARS-CoV-2, a promising alternative to help end the pandemic

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly escalated into the largest global health emergency of the past decades [1]. SARS-CoV-2 is a single-stranded RNA **coronavirus** (see [Glossary](#)) that causes life-threatening respiratory tract infections, and is genetically similar to SARS-CoV and **Middle East respiratory syndrome coronavirus (MERS-CoV)** [2]. Furthermore, novel SARS-CoV-2 variants are frequently being detected and are spreading rapidly around the globe [3].

Several methods for the detection and inhibition of SARS-CoV-2 have been developed, including antigen/antibody-based immunoassays and neutralizing antibodies [4,5]. However, many of these approaches suffer from lack of specificity for emerging SARS-CoV-2 variants [6]. Considering the enormous demand for diagnosis and treatment of SARS-CoV-2 infection, novel alternative techniques with different recognition elements and principles will be necessary to address the current global COVID-19 pandemic.

An **aptamer** is a single-stranded DNA or RNA molecule which can bind to targets by folding into a 3D structure. Aptamers are enriched from a random library of single-stranded nucleic acids by a method called **systematic evolution of ligands by exponential enrichment (SELEX)** [7–9]. SELEX involves progressive selection of aptamers from a large library of random single-stranded oligonucleotides by repeated rounds of partition and amplification [10] (Figure 1A). Compared with antibodies, nucleic acid aptamers can be chemically synthesized and modified with high stability and little batch-to-batch variation. Because they are synthetic molecules, aptamers are cost-effective and have enormous potential in translational research. In addition, aptamers can be easily combined with other cutting-edge technologies such as **CRISPR/Cas**, **nanopore**, and **field-effect transistors** to enhance their performance and application [10,11]. Furthermore, aptamers have been successfully used in the past in therapeutic and

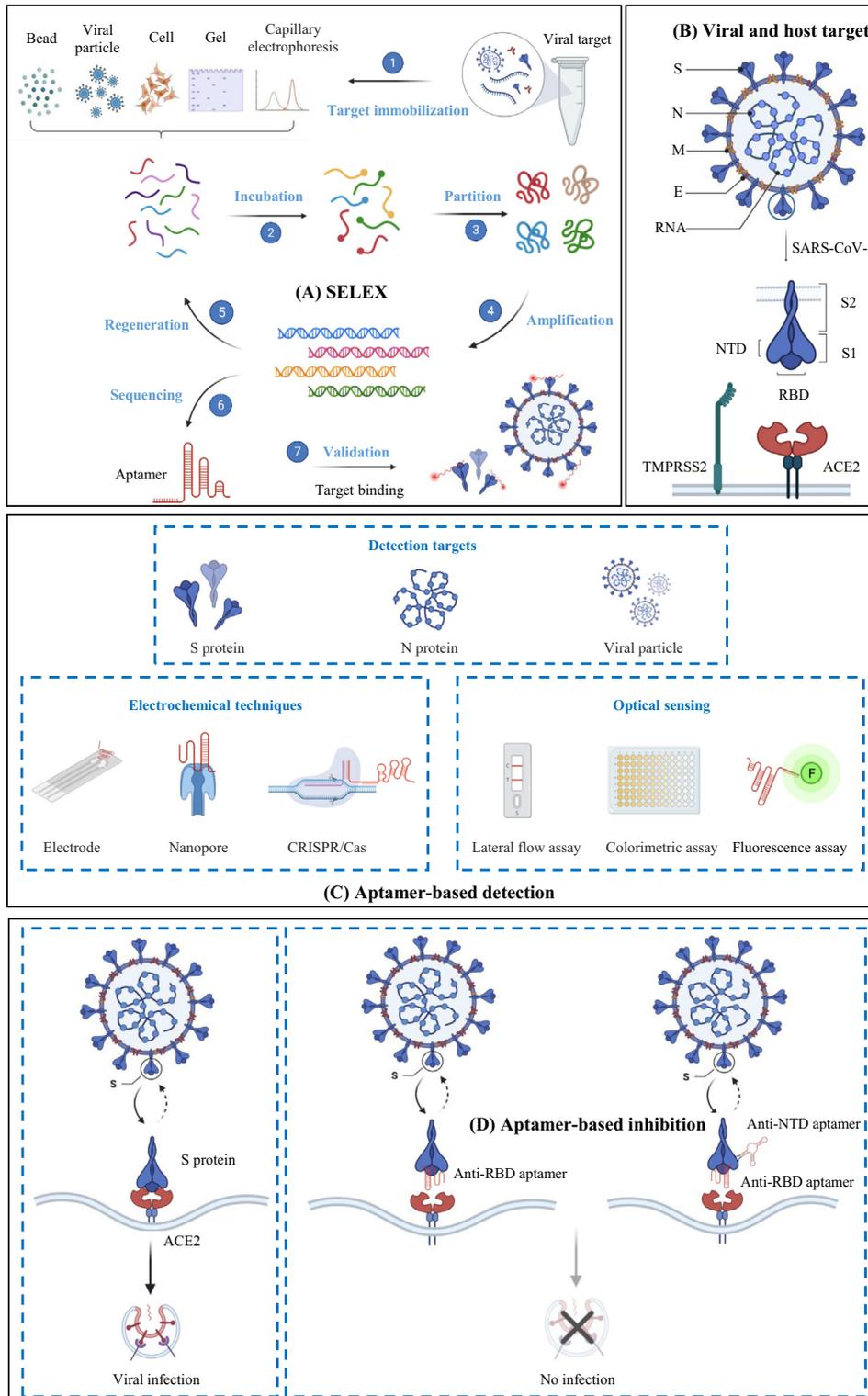
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Glossary

Angiotensin-converting enzyme 2 (ACE2): a protein on the surface of many cell types that acts as the entry receptor for SARS-CoV-2.

Aptamers: single-stranded DNA or RNA oligonucleotides (mostly 20–100 nt) which can form 3D structures and bind to targets with high specificity.

Aptasensor: a type of biosensor in which aptamers constitute the biorecognition element.

Bead-based SELEX: a SELEX method which utilizes beads for the selection of aptamers.

Capillary electrophoresis (CE)-based SELEX: SELEX method which utilizes capillary electrophoresis for the selection of aptamers.

Cell-SELEX: SELEX method using live cells as the target for selection of aptamers.

Coronaviruses: RNA viruses with spikes on their surface, and which cause many human and animal diseases.

CRISPR/Cas: clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein (Cas) are used for precise genome editing and regulation.

Cryo-electron microscopy (cryo-EM): a method to determine 3D structures of molecules and their complexes.

Dissociation constant (K_d): a measure of drug–receptor binding properties.

Electrophoretic mobility shift assay (EMSA)-SELEX: an improved SELEX approach that uses gel electrophoresis to separate and isolate enriched aptamer sequences.

Enzyme-linked oligonucleotide assay (ELONA): a method evolved from ELISA that uses aptamers in quantifying and detecting analytes.

Field-effect transistor: a three-terminal electrical device suitable for biosensing.

G-quadruplex: a noncanonical nucleic acid structure formed by the folding of guanine-rich DNA or RNA.

Half-maximal inhibitory concentration (IC_{50}): the amount of drug needed to inhibit a biological process by 50%.

Limit of detection (LOD): the lowest quantity of an analyte substance that can be determined with a specific analytical test.

Machine learning: a branch of artificial intelligence (AI) which focuses on the use

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(See figure legend at the bottom of the next page.)

diagnostic applications for pathogens including parasites, bacteria, and viruses [12–14]. These traits make aptamers a promising tool for SARS-CoV-2 and its variants. This review provides an overview of recent developments in SARS-CoV-2-specific aptamers with a special focus on their applications in COVID-19 diagnosis and therapy. Updated information regarding aptamers developed against the complete protein or subdomains of spike (S) protein and nucleocapsid (N) protein are summarized. Currently, few studies provide information about the aptamer sequences developed against SARS-CoV-2. This review will therefore also serve as the most comprehensive summary of the available SARS-CoV-2-targeting aptamers. Furthermore, because aptamers have not yet received the same attention as antibodies, we believe that this comprehensive review will help the community to better understand and adopt aptamer techniques to combat SARS-CoV-2 and other emerging viruses.

Success stories in developing aptamers against other viral diseases

Aptamers can potentially bind to any pathogen, making them valuable tools in the fight against a wide range of infectious diseases including many important human viruses (Box 1). Previous outbreaks of respiratory viral diseases include SARS-CoV in 2003, H5N1 influenza (avian flu) in 2004, H1N1 influenza (swine flu) in 2009, and MERS-CoV in 2012. Several aptamers have been developed against these viral infections (Box 2), especially MERS-CoV and SARS-CoV (Table 1), the two closest relatives to SARS-CoV-2. These studies highlight the transformative potential of aptamer-based technologies in viral disease diagnostics and therapeutics, and suggest potential application of similar approaches to SARS-CoV-2.

Aptamers developed against SARS-CoV-2

SARS-CoV-2 is composed of four main structural proteins, namely envelope (E), membrane (M), nucleocapsid (N), and spike glycoprotein (S) [15]. S protein comprises three identical monomers with two distinct subunits, S1 and S2. The S1 subunit is at the outermost layer of the virus, whereas S2 is membrane-bound. In addition, the S1 subunit contains a receptor-binding domain (RBD) and an N-terminal domain (NTD) [16] (Figure 1B). **Transmembrane protease serine 2**

Figure 1. Aptamers for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnosis and treatment. (A) Systematic evolution of ligands by exponential enrichment (SELEX) approaches for the selection of aptamers against viral targets. Step 1 (target immobilization): viral targets can be immobilized on the beads (beads-SELEX), displayed on the viral particles (viro-SELEX), overexpressed by the cells (cell-SELEX), or separated by gel electrophoretic mobility shift assay (EMSA)-SELEX and capillary electrophoresis (CE-SELEX). Step 2 (incubation): incubation of viral targets with a large (10^{14} – 10^{16}) library of random single-stranded oligonucleotides. Step 3 (partition): unbound oligonucleotides are eliminated by washing. Step 4 (amplification): PCR amplification of single-stranded oligonucleotides bound to target viral protein. Step 5 (regeneration): after amplification, the PCR product is purified and double-stranded DNA is separated into single-stranded oligonucleotides. The resulting enriched pool of single-stranded oligonucleotides is used in the next SELEX round for incubation with the viral targets. Step 6 (sequencing): after several rounds of repeated selection (with increasing stringency), the selected pool from the last SELEX round is cloned and sequenced for aptamer identification. Step 7 (validation): further analysis is performed to characterize the interaction of the aptamer with viral targets *in vitro* and *in vivo*. (B) SARS-CoV-2 and host proteins used for aptamers selection. The structural proteins of SARS-CoV-2 include the spike (S), nucleocapsid (N), membrane (M), and envelope (E) proteins. Of the four structural proteins, S protein is most widely used for aptamer selection. The S1 subunit of the S protein contains a receptor-binding domain (RBD) and an N-terminal domain (NTD). The RBD of S protein binds to human angiotensin-converting enzyme 2 (ACE2) on the surface of the host cells. Transmembrane protease serine 2 (TMPRSS2) activates S protein by cleaving at the S1/S2 sites, thus facilitating viral entry into the host cell. These features make S protein an ideal target for aptamer selection. (C) Aptamer-based SARS-CoV-2 detection. Aptamers binding to SARS-CoV-2 targets such as S protein, N protein, or viral particles can be used to develop tests for the diagnosis of SARS-CoV-2. Aptamers targeting SARS-CoV-2 can be combined with various electrochemical and optical sensing methods. (D) Aptamer-based SARS-CoV-2 therapeutics. S protein plays a crucial role in infection, and therefore represents one of the most important targets for coronavirus disease 2019 (COVID-19) therapeutic research. Binding of a monomeric anti-RBD aptamer or an aptamer cocktail (composed of anti-RBD and anti-NTD aptamers) inhibits the binding of S protein to ACE2 on the surface of the host cell, thus preventing viral entry.

of data and algorithms to imitate the way in which humans learn.

Metal organic frameworks (MOFs): organic–inorganic hybrid crystalline porous materials that consist of a regular array of positively charged metal ions surrounded by organic ‘linker’ molecules.

Microfluidic SELEX: a SELEX method that utilizes a microfluidic device for the selection of aptamers.

Middle East respiratory syndrome coronavirus (MERS-CoV): the causative agent of acute respiratory syndrome.

Nanopore: a nanoscale hole embedded in a thin membrane that can be used to detect the change in electrochemical potential when biological molecules pass through the hole for single-molecule biosensing.

Photoelectrochemical (PEC) biosensing: a method that uses photosensitive materials to convert light radiation into electrical signals.

Surface-enhanced Raman spectroscopy (SERS): a Raman spectroscopic method that combines molecular fingerprinting for ultra-high sensitive detection of target molecules.

Surface plasmon resonance (SPR): an optical method for detecting interactions between molecules in real time.

Systematic evolution of ligands by exponential enrichment (SELEX): a method for a progressive selection of aptamers from a large (10^{14} – 10^{16}) library of single-stranded random oligonucleotides (40–100 nt in length) by repeated rounds of partition and amplification.

Transmembrane protease serine 2 (TMPRSS2): a cell-surface protein which activates the S protein of SARS-CoV-2 by cleaving at the S1/S2 sites, thus facilitating viral entry into the host cell.

Viro-SELEX: a virus-based SELEX method for the selection of aptamers.

Virus-like particles (VLPs): virus-derived structures mimicking viruses but which lack viral genes.

Box 1. Aptamers targeting medically important human viruses

To neutralize HIV, several aptamers have been developed against proteins and enzymes involved in HIV infection, including gp120 [58], Gag [59], Rev [60], Tat [61], nucleocapsid [62], reverse transcriptase [63–65], integrase [66], and proteases [67]. For diagnostic applications, highly specific aptamers that bind to HIV Tat protein have been used for the development of aptamer-based biosensors [68,69]. In addition to HIV, cell-SELEX (see Figure 1A in the main text) was employed to generate aptamers that bind to hepatitis C virus (HCV) envelope surface glycoprotein E2 and hepatitis B virus surface antigen (HBsAg) [70,71]. Moreover, a DNA aptamer with high affinity for the envelope glycoprotein (gD) of herpes simplex virus type 1 (HSV-1) was identified, and was shown to inhibit the entry and replication of HSV-1 [72]. RNA aptamers have also been identified which bind strongly to human papillomavirus type 16 (HPV16) virus-like particles (VLPs) [73] and HPV16 E7 oncoprotein [74,75]. In another study, Binning and colleagues [76] developed high-affinity aptamers targeting Ebola-encoded viral protein 35, which can be therefore used as potential inhibitors of Ebola virus. Notably, multiple aptamers were also isolated that bind to vaccinia virus [77,78], thus opening a new direction for the diagnosis and therapy of variola virus (smallpox virus), monkeypox virus, and other orthopoxvirus infections. This is particularly important given the recent multicountry outbreak of monkeypox. Recently, different aptamers have also been developed against tick-borne viral diseases caused by Crimean-Congo hemorrhagic fever virus [79], Dengue virus [80], Japanese encephalitis virus [81], Zika virus [82], and Rift Valley fever virus [83], as well as against food-borne illness caused by norovirus [84].

(TMPRSS2) activates S protein by cleaving at the S1/S2 sites, which facilitates viral entry into the host cell [17]. The RBD serves as the binding site for human **angiotensin-converting enzyme 2 (ACE2)** and mediates host cell recognition [18] (Figure 1B). By playing a critical role in the pathogenesis and infectivity of the virus, S protein is an ideal target for aptamer selection.

Recent studies have identified several S protein-specific aptamers. For example, DNA aptamers targeting the RBD of SARS-CoV-2 S protein were selected by **bead-based SELEX** (Figure 1A) using an ACE2 competition-based selection strategy and a **machine learning** screening algorithm [19]. After rounds of selection, CoV2-RBD-1C and CoV2-RBD-4C aptamers with a low nanomolar **dissociation constant (K_d)** for RBD binding were identified (Table 2) [19]. A similar strategy was used to enrich for another RBD-binding aptamer, CoV2-6C3 (Table 2), that can strongly inhibit RBD binding to ACE2 with a **half-maximal inhibitory concentration (IC_{50})** of 446.68 nM [20]. To improve the binding affinity and stability, a circular bivalent aptamer cb-CoV2-6C3 was constructed by extending a flanking complementary sequence to enable tail-to-tail hybridization

Box 2. Aptamers against respiratory viruses

Many previous studies have reported aptamers targeting respiratory viruses, highlighting their potential in combating viral respiratory diseases. For example, Le and coworkers [85] developed LFAs based on aptamers against H3N2, H5N1, and H9N2 for multiplex strain-specific influenza virus detection. Woo and colleagues [86] used a bead-based SELEX approach to generate RNA aptamers with high affinity for the non-structural protein 1 of influenza virus. Kwon and coworkers [87] used viro-SELEX to generate a pair of aptamers which interact with the hemagglutinin (HA) protein of H3N2. In an integrated study, Lai and colleagues [88] performed **microfluidic SELEX** using H1N1 virus particles conjugated to magnetic beads to identify H1N1-specific aptamers. Aptamers strongly binding to HA1 protein of H5N1 virus have also been identified, and these can be used to inhibit H5N1 [89]. An anti-HA aptamer has also been used to construct a fluorescence aptasensor for H5N1 virus detection [90]. Furthermore, a DNA aptamer targeting the endonuclease domain exhibited cross-protection against H1N1, H5N1, H7N7, and H7N9 [91]. The generation of respiratory syncytial virus (RSV)-selective aptamers using the viro-SELEX strategy was reported, yielding aptamers capable of detecting RSV [92]. The identification of aptamers targeting SARS-CoV and MERS-CoV will aid the rational design of aptamers against SARS-CoV-2. Magnetic bead-based SELEX was employed to screen aptamers against S1 protein of MERS-CoV. DNA aptamer S-19 that binds to MERS S1 can be used to construct SERS- and electrochemical-based biosensors [93] (see Table 1 in the main text). Shum and Tanner have also isolated DNA aptamers against SARS-CoV helicase using a similar magnetic bead-based SELEX strategy. Intriguingly, aptamers identified in this study displayed two different classes of secondary structure **G-quadruplexes** and non-G-quadruplexes. Only the non-G-quadruplex-forming aptamers NG1, NG3, and NG8 (see Table 1 in the main text) could efficiently and specifically inhibit the unwinding activity of SARS-CoV helicase [57]. Moreover, Jang and colleagues selected RNA aptamers against SARS-CoV helicase, yielding an enriched RNA pool from the 15th round of SELEX that can efficiently inhibit the nucleic acid unwinding activity of the viral helicase [94]. Crossreactivity is a noteworthy feature of aptamers because some aptamers against SARS-CoV can be used against SARS-CoV-2. The nucleocapsid (N) protein is the most conserved protein across the coronaviridae families. Cho and colleagues [95] and Ahn and coworkers [96] developed several aptamers against N protein of SARS-CoV (see Table 1 in the main text), and these showed highly specific binding to SARS-CoV-2 N protein *in vitro* [97].

Table 1. Aptamers developed against MERS-CoV and SARS-CoV

Method for aptamer development	Binding target	Aptamer sequence	K_d or K_m (measurement method)	Size (nt)	Application	Refs
Magnetic bead-based SELEX	MERS-CoV S1 protein	S-19: 5'-TGACACCGTACCTGCTCTG CACTTCCTTCACCAGAAACCTGCAC ATCTTCGCCGCGTGAAGCACGCCAA GGGACTAT-3'	Dissociation constant $K_d = 112.18 \pm 3.89$ nM (bead-based fluorescence binding assay)	77	Detection of MERS nanovesicles by surface-enhanced Raman spectroscopy and electrochemical techniques	[93]
Magnetic bead-based SELEX	SARS-CoV helicase	NG1: 5'-GTGTGAGGGTGAGATGTGT GTGTATTTGTC-3' NG3: 5'-AGGTGGGCATGATTGTGTG TTTGTGTCGGT-3' NG8: 5'-ATGTTGGTAGTTGGCTTGTGT TCGTGTGTT-3'	Michaelis constant K_m NG1 = 20.8 nM K_m NG3 = 13.3 nM K_m NG8 = 5.4 nM (ATPase assay)	30	Inhibition of SARS-CoV helicase enzymatic activity	[57]
Bead-based SELEX	SARS-CoV N protein	DNA aptamer 1: 5'-GGATGCGGAAA CTGGCTAATTGGTGAGGCTGGGGCG GTCGTGCA-3'	$K_d = 4.93 \pm 0.30$ nM (ELONA)	44	Detection of N protein by western blot analysis using the aptamer	[95]
SELEX	SARS-CoV N protein	RNA aptamer 1: 5'-UGUCGUUCGCU GUCUJUCUACGUUACGUUACACG GUUGG-3'	$K_d = 1.65 \pm 0.41$ nM (SPR assay)	39	Detection of N protein by aptamer-based chemiluminescence immunosorbent assay and nanoarray aptamer chip	[96]

(Table 2). Compared with the monovalent aptamer ($K_d = 44.78$ nM), the circular bivalent aptamer shows higher binding affinity for the RBD ($K_d = 0.13$ nM) and significantly improved inhibitory activity ($IC_{50} = -32.60$ nM) [20]. In another bead-based SELEX experiment using purified RBD protein, DNA aptamer-1 and -2 were identified that target the RBD with high affinity, and which also block the RBD-ACE2 interaction and prevent host cell infection (Table 2) [21]. Although these three studies used a similar strategy, they resulted in the enrichment of different aptamer sequences owing to differences in the aptamer libraries, stringency of the selection process, sequencing platforms, and bioinformatic analysis pipelines. Comparative sequence and structural analysis of these aptamers will be necessary to identify the best aptamer candidates.

In addition to targeting the RBD, the NTD-specific aptamer SNAP1 was also developed using S protein as the binding target via bead-based SELEX (Table 2). Through binding assays and high-resolution **cryo-electron microscopy (cryo-EM)**, SNAP1 was shown to specifically bind to the NTD but not to the RBD [22]. While RBD is well characterized, the NTD is also an important target for aptamers as the NTD antigenic supersite is exposed [23]. SNAP1 was also shown to bind to the S protein from the B.1.1.7 (Alpha) variant containing several deletions in the NTD [22].

By using the S1 subunit as the selection target, DNA aptamers were screened by a **capillary electrophoresis (CE)-based SELEX** method (Figure 1A). This study identified aptamer nCoV-S1-Apt1 (Table 2) which can neutralize SARS-CoV-2 by binding to the RBD and disrupting the S1-ACE2 interaction [24]. In another study, the S1 subunit has also been used as the target for aptamer selection using a combination of bead-based SELEX and **electrophoretic mobility shift assay (EMSA)-SELEX** (Figure 1A). Aptamers MSA1 and MSA5 were identified with high affinities for S1 and the trimeric S protein of both the wild type (WT) and the B.1.1.7 variant of SARS-CoV-2 (Table 2) [25]. Compared with bead-based SELEX using immobilized protein targets, CE- and EMSA-based SELEX use nonimmobilized targets, thus freeing more epitopes on the protein targets for aptamer binding. Furthermore, the truncated minimal sequences of MSA1 and MSA5, namely MSA1T and MSA5T (Table 2), were linked by a polythymidine (poly-T) linker to generate the dimeric aptamers DSA1N1, DSA5N5, and DSA1N5. Notably, the dimeric aptamers show significantly enhanced affinity for the trimeric S protein (Table 2). In

Table 2. Representative aptamers developed against SARS-CoV-2^a

Method for aptamer development	Binding target	Aptamer sequence	K_d (method for K_d measurement)	Size (nt)	Application	Refs
Bead-based SELEX using RBD as the target and ACE2 as the competitor	Receptor-binding domain (RBD)	CoV2-RBD-1C: 5'-CAGC ACCGACCTTGTGCTTTG GGAGTGTGGTCCAA GGGCGTTAATGGACA-3'	5.8 ± 0.8 nM (flow cytometric analysis)	51	Electrochemical aptasensor, SERS-based aptasensor, and SPR aptasensor for detection	[19]
		CoV2-RBD-4C: 5'-ATCC AGAGTGACGCAGCATTT CATCGGGTCCAAAAG GGGCTGCTCGGGATT GCGGATATGGACA CGT-3'	19.9 ± 2.6 nM (flow cytometric analysis)	67	Colorimetric assay and thermophoretic assay for detection	
Bead-based SELEX using RBD as the target and ACE2 as the competitor	RBD	CoV2-6C3: 5'-CGCAGC ACCCAAGAACAAGGA CTGCTTAGGATTGCG ATAGGTTCCG-3'	44.78 ± 9.97 nM (flow cytometric analysis)	46	Neutralization against SARS-CoV-2 by blocking the RBD-ACE2 interaction	[20]
		cb-CoV2-6C3: 5'-CGTA AATCAG TCACGCAGC ACCCAAGAACAAGGACT GCTTAGGATTGCGAT AGGTTTCGGTGACTGA TTTACGCGCAGCACC CAAGAACAAGGACTG CTTAGGATTGCGATA GGTTCCG-3'	0.13 ± 0.04 nM (flow cytometric analysis)	118		
Bead-based SELEX using RBD as the target	RBD	Aptamer-1: 5'-ATCCAG AGTG ACGCAGCATCG AGTGCGTTGTTTGTG ATGTAGGGTTCCGGT CGTGGGTTGGACACG GTGGCTTAGT-3'	6.05 ± 2.05 nM (flow cytometric analysis)	76	Neutralization of SARS-CoV-2 by blocking the RBD-ACE2 interaction	[21]
		Aptamer-2: 5'-ATCCAG AGTG ACGCAGCAATT ACCGATGGCTTGTGT GTAATGTAGGGTTCC GTCGGATTGGACACG GTGGCTTAGT-3'	6.95 ± 1.10 nM (flow cytometric analysis)			
Magnetic bead-based SELEX using SARS-CoV-2 S protein as the target and MERS-CoV S1 and SARS-CoV S1 as the competitors	N-terminal domain (NTD)	SNAP1: 5'- TCGCTCTTT CCGCTTCTTCGCGGTCA TTGTGCATCTGACT GACCCTAAGGTGCGA ACATCGCCCCGCGTAA GTCCGTGTGTGCGAA-3'	NTD: 60.35 ± 1.61 nM S1: 39.32 ± 0.12 nM (BLI assay)	86	LFA and ELISA for detection	[22]
Magnetic bead-based SELEX using S1 protein as the target	S1	XN-268s: 5'-GGGGTG GGGTAGTGGTATGGA GCG-3'	4.26 nM (SPR assay)	24	Electrochemical detection of SARS-CoV-2	[32]
Capillary electrophoresis (CE)-based SELEX using S1 protein as the target	RBD, S1	nCoV-S1-Apt1: 5'-CCGC AGGCAGCTGCCATTAGT CTCTATCCGTGACGG TATG-3'	RBD: 1.56 ± 0.22 μM S1: 0.327 ± 0.016 nM (capillary electrophoresis)	40	Neutralization of SARS-CoV-2 by blocking the RBD-ACE2 interaction	[24]
Magnetic bead-based and EMSA-based SELEX using S1 protein as the target	S1, RBD, trimeric S protein	MSA1: 5'-TTACGTCAAG GTGTCACTCCCACTTT CCGGTTAATTTATGC TCTACCGTCCACCT ACCGGAAGCATCTCT TTGGCGTG-3'	S1: 1.8 ± 0.4 nM RBD: 3.1 ± 0.8 nM Trimeric S: 19.8 ± 2.4 nM (dot-blot assay)	79	Colorimetric sandwich assay for detection	[25]
		MSA5: 5'-TTACGTCAAG GTGTCACTCCACGGGT	S1: 2.7 ± 0.2 nM RBD: 4 ± 0.6 nM			

Table 2. (continued)

Method for aptamer development	Binding target	Aptamer sequence	K_d (method for K_d measurement)	Size (nt)	Application	Refs
		TTGGCGTCGGGCCTG GCGGGGGGATAGTGC GGTGAAGCATCTCT TTGGCGTG-3'	Trimeric S: 5.6 ± 0.6 nM (dot-blot assay)			
Truncated minimal sequences of the aptamers MSA1 and MSA5	S1, trimeric S protein	MSA1T: 5'-TTCCGGTTA ATTTATGCTCTACCCGT CCACCTACCGGAA-3'	Trimeric S: 11.9 ± 1.8 nM S1: 2.8 ± 0.5 nM (dot-blot assay)	39	Electrochemical impedance sensor for detecting viral particles	[26]
		MSA5T: 5'-ACGGGTTTG GCGTCGGGCCTGGCG GGGGGATAGTGC GGT-3'	Trimeric S: 3.4 ± 0.6 nM S1: 10.1 ± 1.4 nM (dot-blot assay)			
Dimeric aptamers were generated by linking the MSA1T and MSA5T with a polythymidine (poly-T) linker		DSA1N1: 5'-TTCCGGTTA ATTTATGCTCTACCCGT CCACCTACCGGAATTTTT TTTTTTTTTTTTTTTTTTTT TTTTTTTCCGGTTAATT TATGCTCTACCCGTC CACCTACCGGAA -3'	Trimeric S: 1.9 ± 0.1 nM (dot-blot assay)	108		
		DSA5N5: 5'-ACGGGTTTG GCGTCGGGCCTGGCG GGGGGATAGTGC TTTTTTTTTTTTTTTTTTTT TTTTTTTTTACGGGTT TGGCGTCGGGCCTGG CGGGGGGATAGTGC GT-3'	Trimeric S: 0.65 ± 0.07 nM (dot blot assay)			
		DSA1N5: 5'-TTCCGGTTA ATTTATGCTCTACCCGT CCACCTACCGGAATT TTTTTTTTTTTTTTTTTTTT TTTTTTTTTACGGGTTTGG CGTCGGGCCTGGCGG GGGGATAGTGC GGT-3'	Trimeric S: 0.12 ± 0.02 nM S1: 0.51 ± 0.04 nM (dot-blot assay)			
Five parallel one-round EMSA-based SELEX using a pre-enriched pool [25] with S protein of the original SARS-CoV-2 and its B.1.1.7, B.1.351, P.1, and B.1.429 variants	Trimeric S proteins of the original SARS-CoV-2 and its B.1.1.7, B.1.351, P.1, B.1.429, B.1.617.2, and B.1.1.529 variants	MSA52: 5'-TTACGTCAA GGTGCTACTCCGTAG GGTTTGGCTCCGGGC CTGGCGTCGGTCGTC TCTCGCGAAGCATCTCT TTGGCGTG-3'	Trimeric S (WT): 3.6 ± 0.4 nM Trimeric S (B.1.1.7): 3.8 ± 0.2 nM Trimeric S (B.1.351): 8.5 ± 0.8 nM Trimeric S (P.1): 10.2 ± 1.4 nM Trimeric S (B.1.429): 3.8 ± 0.6 nM Trimeric S (B.1.617.2): 3.7 ± 0.4 nM Trimeric S (B.1.1.529): 6.2 ± 0.6 nM (dot blot assay)	79	Sandwich assay for the detection of pseudotyped lentiviruses expressing the original SARS-CoV-2 and the B.1.1.7, B.1.351, P.1 and B.1.617.2 variants	[27]
Bead-based SELEX using trimeric S protein as the target	Trimeric S protein	ST-6: 5'-AGCAGCACAGA GGTCAGATGAGGGCA TCAAAGGGGGGAGGG CGGGTGGATTGGATG CCGACCTATGCGTGC TACCGTGAA-3'	35.62 ± 2.65 nM (BLI assay)	80	Inhibition of SARS-CoV-2 induced inflammation by blocking the S-TLR4 interaction	[31]
		ST-6-1: 5'-AGGGCATCA AAGGGGGGAGGGCGG	79.44 ± 1.29 nM (BLI assay)	40		

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Table 2. (continued)

Method for aptamer development	Binding target	Aptamer sequence	K_d (method for K_d measurement)	Size (nt)	Application	Refs
		GTGGATTGGATGCCGA -3'				
		ST-6-2: 5'-GGGGAGGGC GGGTGGATTGGATGC CGA -3'	80.22 ± 0.82 nM (BLI assay)	27		
Viro-SELEX using active pseudotyped SARS-CoV-2 with S protein as the target and counterselection against UV inactivated pseudotyped SARS-CoV-2, pseudotyped SARS-CoV-1 with S protein and pseudotyped H5N1	Active pseudotyped SARS-CoV-2	SARS2-AR10: 5'-CCCG ACCAGCCACCATCAG CAACTCTTCCGCGTCCA TCCCTGCTG-3'	79 ± 28 nM (ELONA)	45	Nanopore incorporation for the detection of SARS-CoV-2 virions	[28]
Magnetic bead-based SELEX using nucleocapsid (N) protein as the target	Nucleocapsid (N) protein	A48: 5'-GCTGGATGTCG CTTACGACAATATTCCTT AGGGGCACCGCTACA TTGACACATCCAGC -3'	A48: 0.49 ± 0.05 nM (SPR assay)	58	ELISA, gold nanoparticle immunochromatographic strip assay, SPR aptasensor, microfluidic chip, and CRISPR/Cas12a-derived aptasensor for the detection of N protein	[29]
		A58: 5'-GCTGGATGTCA CCGGATTGTCCGACA TCGGATTGTCTGAGT CATATGACACATCCAGC -3'	A58: 0.70 ± 0.06 nM (SPR assay)			
		A61: 5'-GCTGGATGTTG ACCTTTACAGATCGG ATTCTGTGGGGCGTT AAACTGACACATCCA GC-3'	A61: 2.74 ± 0.08 nM (SPR assay)			
		A15: 5'-GCTGGATGTTT ATGCTGGCAAATTC CTTAGGGGCACCGTT ACTTTGACACATCCA GC-3'	A15: 4.38 ± 0.06 nM (SPR assay)			

^aNote: B.1.1.7 (the UK variant; Alpha), B.1.351 (the South Africa variant; Beta), P.1 (the Brazil variant; Gamma), B.1.429 (the California variant; Epsilon), B.1.617.2 (the Indian variant; Delta), and B.1.1.529 (Omicron).

addition to the WT S protein, the dimeric aptamer DSA1N5 could also bind to the S protein of the B.1.1.7 ($K_d = 0.29$ nM) and B.1.617.2 ($K_d = 0.48$ nM) variants [26].

S protein forms trimeric structures on the viral envelope, and using only a truncated form containing either the RBD or the NTD, or its monomeric form, would therefore lose a significant amount of quaternary structure that could be used by aptamers for recognition. Moreover, aptamers would be more sensitive to conformational changes of S protein caused by mutations in SARS-CoV-2 variants. For example, MSA1 and MSA5 binding to trimeric S proteins of different SARS-CoV-2 variants exhibited decreased affinity for some strains [27]. To recognize fully functional S protein, a parallel one-round EMSA-based SELEX (Figure 1A) was performed using a pre-enriched aptamer pool [25] with trimeric S protein of the WT virus and its B.1.1.7, B.1.351, P.1, and B.1.429 variants. This study led to the discovery of MSA52, a universal aptamer that can bind to the trimeric S proteins of the WT virus and diverse variants of concern, including B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.429 (Epsilon), B.1.617.2 (Delta), and very recently

B.1.1.529 (Omicron) (Table 2) [27]. The MSA52 aptamer that recognizes all S proteins has the potential to target future SARS-CoV-2 variants.

Notably, the purified trimeric S protein in its soluble form used in this study is significantly different from the membrane-bound S protein on the viral envelope. To better mimic the native state of S protein, a lentivirus-based pseudotyped SARS-CoV-2 containing S protein was employed to develop virus-based SELEX (**viro-SELEX**) (Figure 1A), yielding aptamer SARS2-AR10 (Table 2) that binds to active SARS-CoV-2 but not to UV-inactivated SARS-CoV-2 or other viruses such as 229E coronavirus, SARS-CoV, or influenza H5N1 [28].

In addition to S protein, four DNA aptamers that specifically bind to the N protein of SARS-CoV-2 were derived by bead-based SELEX. After optimization and truncation, A48 was identified as the strongest binding aptamer to N protein by **surface plasmon resonance (SPR)** assay (Table 2) [29]. N protein serves multiple purposes, such as packaging the RNA genome and regulating viral RNA synthesis [30]. Most studies have so far focused on the development of aptamers against S protein, and more research will be necessary to develop and explore the potential of aptamers targeting other viral structural proteins or non-structural proteins.

To determine the aptamer with best potential for diagnosis and therapy of COVID-19, the binding strength (affinity), specificity, and sensitivity of aptamers through various biochemical and biophysical techniques will need to be systemically compared in future studies. To assess the affinity of aptamers for viral targets, various bioanalytical methods can be employed, including flow cytometric analysis [19–21], biolayer interferometry (BLI) [22,31], capillary electrophoresis [24], dot-blot assay [25–27], and SPR [29,32] (Table 2). The specificity of aptamers can be delineated by comparing discriminatory binding against similar viral proteins such as proteins from SARS-CoV, MERS-CoV, and SARS-CoV-2 variants.

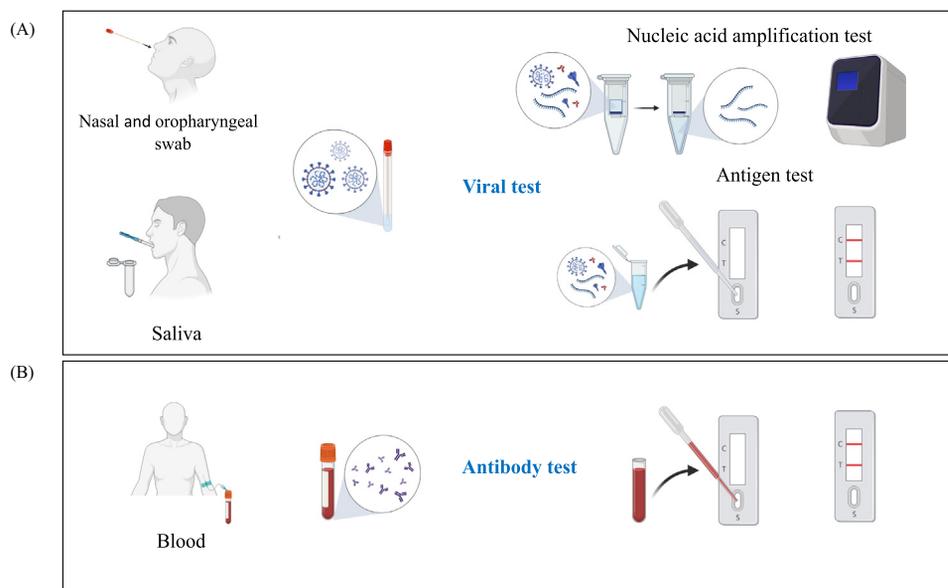
Aptamer-based detection methods for SARS-CoV-2 diagnostics

Real-time reverse transcription PCR (RT-PCR) is recommended for the detection of SARS-CoV-2 RNA in respiratory samples such as nasal or oropharyngeal swabs and saliva specimens (Figure 2A) [14]. However, RT-PCR is time-consuming and costly, and also requires sophisticated equipment and well-trained professionals. In addition to the detection of viral nucleic acid, the presence of SARS-CoV-2 in respiratory specimens can also be quickly assessed by antigen testing for viral proteins (Figure 2A) [14]. Although antigen testing is less sensitive, its rapid results make it an appropriate tool for point-of-care testing (POCT). Antibody testing is designed to identify SARS-CoV-2-specific IgM/IgG antibodies from current or past infections (Figure 2B). Serological testing is recommended for epidemiological and surveillance studies [14].

A rapid detection test for SARS-CoV-2 to monitor virus spread and containment is urgently required. To date, several aptamer-based detection methods have been developed with remarkable success, including **enzyme-linked oligonucleotide assay (ELONA)**, aptamer-based lateral flow assay (LFA), and aptamer-based biosensors (**aptasensors**) [12,14]. Compared with protein biorecognition elements, nucleic acid aptamers can be easily coupled to different electrochemical and optical sensing techniques for ultrasensitive, rapid, and portable testing. An illustration outlining the methods of aptamer-based SARS-CoV-2 detection, by either electrochemical or optical sensing, is presented in Figure 1C.

Electrochemical measurement

Various aptamer-based electrochemical methods have been successfully used to detect SARS-CoV-2 viral particles or proteins in clinical specimens. Aptamers against S protein are most widely



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Figure 2. Current methods for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detection. (A) Viral testing. The presence of SARS-CoV-2 in the respiratory samples, such as nasal and oropharyngeal swabs or saliva specimens, can be assessed by analyzing viral nucleic acid and antigens. Nucleic acid amplification testing of conserved gene sequences in the viral genome can be performed by real-time reverse transcription (RT)-PCR. Viral antigens can be detected directly by a rapid antigen (lateral flow) test, thereby directly verifying that the sample contains SARS-CoV-2. (B) Antibody testing. Virus infection stimulates the production of specific antibodies. The presence of IgM and IgG antibodies against SARS-CoV-2 in blood provides an indirect indicator of current or previous infection.

used, and these are often conjugated to gold-coated electrodes in electrochemical aptasensors. For example, aptamer CoV2-RBD-1C (Table 2) targeting the RBD was immobilized on screen-printed carbon electrodes coated with gold nanoparticles for electrochemical detection of SARS-CoV-2 S protein with a **limit of detection (LOD)** of 1.30 pM (66 pg/ml). The aptasensor shows selectivity for S proteins of SARS-CoV and SARS-CoV-2, but not for MERS-CoV [33]. The same CoV2-RBD-1C aptamer was then used to develop an aptamer-based electrochemical assay based on electrodes made using a children's toy, Shrinky Dink™. The low-cost electrodes can detect S1 protein down to 1 ag/ml in saliva [34]. Similarly, aptamer XN-268s targeting S1 protein [32] and dimeric aptamer DSA1N5 targeting trimeric S protein [26] were immobilized on nanochannels or gold electrodes for electrochemical detection of SARS-CoV-2 with high sensitivity and specificity (Table 2).

In addition to conventional electrochemical techniques, **photoelectrochemical (PEC) biosensing** has also been used to detect SARS-CoV-2. The PEC aptasensor is designed based on a signal-off strategy by engineering aptamers targeting SARS-CoV-2 with photoactive materials. For example, a **metal organic framework (MOF)**-based PEC aptasensor for the detection of SARS-CoV-2 S protein was constructed using aptamer CoV2-RBD-1C, and achieved high sensitivity (a linear response range of 0.5–8 µg/ml and a detection limit of 72 ng/ml) [35]. S protein binding to the aptamer immobilized on the surface of photoactive materials blocks photoelectron transfer, resulting in decreased photocurrent. Moreover, the same aptamer CoV2-RBD-1C was used with a graphitic carbon nitride (gC₃N₄)-cadmium sulfide quantum dot (CdS QD) nanocomposite to construct another signal-off PEC aptasensor for the measurement of RBD (with a detection range of 0.5–32.0 nM and a LOD of 0.12 nM) [36].

Nanopore and CRISPR/Cas systems coupled with aptamers are emerging as powerful techniques for the rapid and portable detection of SARS-CoV-2 with ultra-high sensitivity (at the single-molecule level). In particular, aptamer SARS2-AR10 (Table 2) was incorporated into a solid-state nanopore to allow highly sensitive detection of SARS-CoV-2 with a LOD of 1×10^4 copies/ml. The aptamer–nanopore system can directly quantify SARS-CoV-2 in saliva without any sample pretreatment [28]. Furthermore, by using aptamer A48 (Table 2) targeting N protein of SARS-CoV-2, a CRISPR/Cas12a-derived electrochemical aptasensor was constructed [37]. Notably, the aptasensor can obtain results within 30 minutes without large instruments, thus providing a promising alternative approach for portable and fast detection of COVID-19.

Optical sensing

A wide variety of aptamer-based optical techniques have also been reported in which aptamers are often coupled to nanomaterials. Among these methods, colorimetric assays show high promise for the detection of SARS-CoV-2 owing to their relatively low cost. For example, gold nanoparticles coupled with CoV2-RBD-4C aptamer (Table 2) have been used to develop a colorimetric assay for the detection of S protein (16 nM and higher concentrations) and inactivated SARS-CoV-2 virus (3540 genome copies/ μ l and higher concentrations). The colorimetric test can be performed using an inexpensive spectrophotometer to examine the color change in the presence of S protein [38]. In parallel, aptamer SNAP1 (Table 2) targeting the NTD was also used in a LFA and ELISA to detect SARS-CoV-2 S protein and UV-inactivated SARS-CoV-2 virus [22].

Fluorescence detection is another commonly used method to visualize viral infection, and is more sensitive and accurate than most colorimetric systems. In particular, CoV2-RBD-4C labeled with rhodamine 6G dye was attached to gold nanostars for fluorescence detection of S protein and viral particles by distance-dependent nanoparticle surface energy transfer spectroscopy, achieving a LOD as low as 130 fg/ml for S protein and 8 particles/ml for virus [39]. In another study, Cy5-labeled CoV2-RBD-4C aptamer was used to construct an aptamer-based thermophoretic assay for direct detection of SARS-CoV-2 viral particles. Under a temperature gradient induced by localized IR laser heating, aptamer binding to the S protein and polyethylene glycol (PEG)-enhanced thermophoretic accumulation of viral particles were observed. The fluorescence intensity of the enriched aptamer and virus complex correlated linearly with the level of S protein on the viral particles. Using a pseudotyped lentivirus model, a LOD of \sim 170 particles/ μ l (26 fM spike protein) was achieved in 15 minutes [40].

In addition, the CoV2-RBD-1C aptamer (Table 2) was used as a receptor and immobilized on a gold nanopopcorn surface to construct a **surface-enhanced Raman scattering (SERS)**-based aptasensor for fluorescence detection of SARS-CoV-2. After the RBD binds to the aptamer, the S protein-bound aptamers move away from the gold nanopopcorn surfaces, reducing the Raman peak intensity of Cy3 reporters. The Cy3 Raman intensity decreased as the amount of the S protein increased, allowing sensitive detection of SARS-CoV-2 with a LOD of less than 10 plaque-forming units (PFU)/ml in 15 minutes [41]. Moreover, the CoV2-RBD-1C aptamer was also used to construct a dual-mode SERS-based aptasensor that can accurately diagnose and distinguish between SARS-CoV-2 and influenza A/H1N1. The same sensing principle was employed for monitoring changes in Cy3 SERS signals. Binding of the SARS-CoV-2 virus to the aptamer was sensed by SERS, resulting in a LOD of 0.78 PFU/ml for SARS-CoV-2 [42]. The same CoV2-RBD-1C aptamer was further used to construct a colloidal SERS-based aptasensor, enabling operation on a handheld Raman spectrometer for POCT. The method is one-step and rapid (7 minutes), has high sensitivity and specificity, and distinguishes SARS-CoV-2 from other viral respiratory viruses including influenza virus, adenovirus, respiratory syncytial virus, and Newcastle disease virus [43]. Apart from aptamers targeting S protein, a recent study has tested

the anti-N protein aptamers A58 and A61 (Table 2) in a microfluidic chip with femtoliter-sized wells for fluorescence detection of N protein, achieving a LOD of 33.28 pg/ml [44].

In addition to using the fluorescent dyes mentioned earlier, label-free optical approaches such as SPR provide simple methods for SARS-CoV-2 detection. For instance, the aptamer CoV2-RBD-1C (Table 2) was immobilized on a short PEG interface on gold nanofilm deposited on a D-shaped plastic optical fiber probe, and S protein binding was monitored by exploiting the very sensitive SPR phenomenon, achieving a LOD of 37 nM for S protein [45]. A SPR aptasensor has also been constructed for the detection of N protein of SARS-CoV-2 by using niobium carbide MXene quantum dots to anchor aptamer A58 (Table 2), resulting in a LOD of 4.9 pg/ml for N protein [46]. Another label-free biosensing method was developed, called photonic resonator interferometric scattering microscopy (PRISM), that uses the aptamer SARS2-AR10 (Table 2) and a photonic crystal as the transducer substrate, and allowed real-time visualization and mass quantification of SARS-CoV-2 virions (LOD 1×10^3 copies/ml) [47]. Although the aforementioned SERS-based, SPR-based, or PRISM-based aptasensors provide promising detection results compared with LFA and ELISA, detecting the optical signals requires advanced, specialized, and costly equipment.

Aptamers constitute a promising next-generation solution for viral diagnosis. We therefore expect that aptamer-based biosensing methods will be developed as rapid, sensitive, and cost-effective tests for diagnosing COVID-19.

Aptamers against SARS-COV-2 with neutralization activity

New neutralizing agents against SARS-CoV-2 and associated mutant strains are urgently needed for the treatment and prophylaxis of COVID-19. Compared with therapeutic antibodies, neutralizing aptamers show lower immunogenicity and are more easily engineered and chemically modified [10]. S protein represents one of the most important targets for COVID-19 therapeutic research. Most therapeutic aptamers can efficiently inhibit virus infection by strongly binding to the RBD domain and disrupting S protein binding to the ACE2 receptor. Neutralizing DNA aptamers blocking the RBD-ACE2 interaction include CoV2-6C3 and cb-CoV2-6C3 [20], aptamer-1 and aptamer-2 [21], and nCoV-S1-Apt1 [24] (Table 2). Although DNA aptamers are gaining popularity due to faster selection time and higher stability, RNA aptamers form more diverse and intricate 3D structures. A serum-stable (2'-fluoropyrimidine-modified) RNA aptamer RBD-PB6 with neutralization activity against SARS-CoV-2 was reported which could efficiently block viral uptake through binding to the RBD with nM affinity and blocking its interaction with the ACE2 receptor [48]. RBD-PB6 bound strongly to S protein from variants of concern, including the Beta ($K_d = 38$ nM) and Alpha ($K_d = 12$ nM) variants. This RNA aptamer showed high specificity for SARS-CoV-2 and could neutralize **virus-like particles (VLPs)** expressing the WT ($IC_{50} = 200$ nM) or D614G spike variant ($IC_{50} = 110$ nM) with no crossreactivity with VLPs pseudotyped with either MERS or SARS-CoV-1 S proteins. Furthermore, the trimeric form of RBD-PB6 aptamer showed the strongest neutralizing effect against the original SARS-CoV-2 virus ($IC_{50} = 46$ nM) without apparent cytotoxicity [48]. Recently, 2'-fluoro-arabino nucleic acid (FANA) xeno-nucleic acid (XNA) aptamers with resistance to degradation were generated by SELEX targeting the RBD of SARS-CoV-2 S protein. These newly developed FANA aptamers bind with low nM affinity to the RBD and block ACE2 binding [49]. Considering that the RBD frequently mutates, changes in the RBD of new SARS-CoV-2 variants might impair the antiviral efficacy of some anti-RBD aptamers. Therefore, treatment with a cocktail of aptamers may prevent the emergence of escape mutants. To investigate this option, Sun and colleagues recently developed a spherical cocktail of neutralizing aptamers by anchoring aptamers CoV2-RBD-1C, CoV2-RBD-4C, and CoV2-6C3 (Table 2) to the same gold nanoparticle. This spherical cocktail of neutralizing

aptamer–gold nanoparticles showed high affinity for the RBD, with a K_d in the picomolar range, and also showed strong neutralization activity against WT pseudotyped SARS-CoV-2 ($IC_{50} = 207.7 \pm 17$ fM), as well as against pseudotyped SARS-CoV-2 N501Y ($IC_{50} = 15.6 \pm 3.9$ pM), D614G ($IC_{50} = 1.2 \pm 0.1$ pM), K417N:E484K:N501Y ($IC_{50} = 89.9 \pm 10.9$ pM), and Omicron ($IC_{50} = 35.9 \pm 7.6$ pM) variants [50,51]. More importantly, the attachment to gold nanoparticles not only inhibits SARS-CoV-2 infection by blocking binding to ACE2 but also improves the *in vivo* stability of the aptamers by deactivating nucleases [50]. An illustration outlining aptamer-based therapeutics against SARS-CoV-2 infection by directly inhibiting S–ACE2 binding is shown in Figure 1D. Unlike monomeric aptamers, multivalent aptamers can simultaneously block distinct epitopes on S protein that are involved in the interaction with ACE2 and viral uptake.

In addition to inhibiting S-ACE2 binding, aptamer ST-6 screened against S was also shown to be able to block the S-TLR4 (Toll-like receptor 4) interaction and selectively inhibit SARS-CoV-2-induced inflammation. TLR4 is activated by interacting with S protein, which contributes significantly to the pathogenesis of SARS-CoV-2 [31]. Aptamer ST-6 and its two truncated versions (ST-6-1 and ST-6-2) (Table 2) display strong anti-inflammatory potency against SARS-CoV-2 and might be used in COVID-19 therapeutics.

These recent studies show the potential of aptamers to inhibit the binding of SARS-CoV-2 to human receptors, thus preventing infection. A similar aptamer-mediated blocking strategy can be used to neutralize SARS-CoV-2 by targeting other viral targets involved in viral entry, replication, assembly, and release. These aptamers could be added to the array of therapeutic molecules, such as monoclonal antibody therapies, that are currently being evaluated for their potential to inhibit SARS-CoV-2 infection.

Concluding remarks and future perspectives

Recent work has led to great advances in the identification and analysis of aptamers targeting SARS-CoV-2. These analyses have also revealed several limitations and challenges (see Outstanding questions).

SARS-CoV-2 mutates quickly, and new variants such as Omicron are spreading rapidly around the globe, but the aptamer selection process is long and tedious. Furthermore, not many laboratories have the facilities and resources to perform SELEX experiments. However, it might not be necessary to repeat SELEX for each new variant. Instead, bioinformatic tools can be employed to repurpose already known aptamers targeting the WT virus for use against SARS-CoV-2 variants. *In silico* approaches can be employed for the identification of novel aptamers against SARS-CoV-2 and its emerging variants. Currently, SELEX combined with high-throughput sequencing has been widely used to screen aptamers, leading to thousands of potential candidate sequences [22,25]. Although aptamer selection has generated vast troves of sequencing data, most sequences have not yet been fully utilized. To prioritize aptamer candidates with high affinity, further optimization such as shortening and sequence modifications is necessary [26,31,48]. Clustering algorithms revealing highly populated clusters or motifs from different enriched SELEX pools are expected to identify conserved domains and reduce the time required for the design and discovery of aptamers against SARS-CoV-2 variants [52,53].

Despite tremendous success in developing aptamers against SARS-CoV-2, only a few have reached the market so far. To our knowledge, only two aptamer-based methods are now in clinical trials, namely the saliva-based COVID-19 DNA Aptamer Test (Clinical trials.gov identifier NCT04974203) and the use of aptamers for the treatment of COVID-19 (NCT05293236), which is currently in Phase 1 of clinical evaluation. One potential bottleneck for translation to

Outstanding questions

How can predictive sequence and structural information obtained from *in silico* approaches, such as comparative sequence/structural analysis, be applied to the discovery and design of aptamers against SARS-CoV-2 and its variants?

How can previously reported aptamer data be extracted to facilitate the development and application of aptamers against SARS-CoV-2 and its current and future variants?

Do the current targets used for the development of aptamers against SARS-CoV-2 pose a problem in clinical application? How can viral targets in their natural state with an intact structure be used for aptamer selection, and how can the aptamer selection process be expanded to include host factors?

Which detection principles would give best performance when combined with aptamers? How can these detection approaches be combined with aptamers for the development of novel diagnostic tests for SARS-CoV-2?

How can we improve the efficacy of aptamers with increased and broader neutralization activity against SARS-CoV-2 and its variants?

As many of the aptamers were tested only *in vitro*, how can we improve the performance of aptamers, such as stability, affinity, and delivery efficiency, for *in vivo* clinical applications?

the clinic is that the mechanisms of actions of the aptamers are not yet completely clarified. Structural analysis could provide more information about the binding epitope in the aptamer targets. Binding-domain information allows a mechanistic understanding of how aptamers function at the molecular level by addressing the fundamental question of how they bind to their targets. Structural data collected from published aptamers could be used for aptamer screening and repurposing to target SARS-CoV-2 and its variants. However, structural information on the aptamers and viral targets is scarce. Therefore, bioinformatic tools for secondary and tertiary structure prediction, as well as docking simulation of aptamers and their viral targets, can be employed to provide valuable guidelines for the design of novel antiviral agents [53].

Universal aptamers exhibiting cross-protection against infections by SARS-CoV-2 and its variants could be developed by targeting conserved viral epitopes. However, one of the challenges of this approach is that the neutralization epitopes might be mutated in new variants. Future work will therefore be necessary to evaluate whether any of these aptamers can neutralize newly emerging SARS-CoV-2 variants. Understanding the mechanism by which aptamers interact with their binding epitopes on SARS-CoV-2 will support the design of strain-specific or broad-spectrum therapeutics.

Conformation differences between the native and recombinant viral proteins represent a challenge in developing aptamer-based technologies for clinical applications. This is particularly the case for S protein which forms a trimeric structure on the viral envelope [3,54]. Although some studies have tried to utilize pseudoviruses displaying the trimeric form of S protein as the selection target [28], this could still lead to the selection of aptamers with suboptimal performance in the clinic. The development of reliable aptamers is important for successful clinical translation. In addition, most current aptamers were screened against viral proteins, and very few aptamers targeting host factors crucial for SARS-CoV-2 pathogenesis were selected. Importantly, ACE2-targeting aptamers might block RBD binding, thus serving as broad-spectrum inhibitors of multiple coronaviruses that utilize ACE2 for entry. Targeting TMPRSS2 protease activity through aptamers might also prevent SARS-CoV-2 entry, and could be a potential treatment option for COVID-19. However, potential cellular side-effects and cytotoxicity will need to be addressed for aptamers targeting host factors.

To develop highly selective and ultrasensitive aptasensors for SARS-CoV-2, aptamers can be easily combined with conventional detection techniques including, but not limited to, fluorescence, electrochemical techniques, and cutting-edge detection systems such as CRISPR/Cas, field-effect transistors, or nanopores [28,55,56]. Aptasensors inspired by these endeavors would open the possibility of developing high-throughput, super-sensitive (single-molecule level), and portable tests for SARS-CoV-2 that would have several advantages over existing antibody-based detection methods.

Given that SARS-CoV-2 S protein adopts a trimeric structure on the viral envelope, aptamer multimerization would improve their binding affinity for the viral target as well as their neutralization efficacy [48,50]. Scaffolding aptamers in their dimeric or trimeric forms have increased binding avidity and increased inhibitory effects against SARS-CoV-2 [48]. To obtain cross-neutralizing activity against SARS-CoV-2 and its variants, an aptamer cocktail of multivalent and multisite binding aptamers could be further investigated.

Another major limitation to the clinical application of aptamers developed *in vitro* is that they might not be equally stable *in vivo*. Successful clinical translation also requires extremely high stability of the aptamer. Therefore, 2'-fluoropyrimidine modification or other nucleic acid mimics can be used

to increase the chemical stability of the aptamers and their resistance to nucleases [48]. Moreover, capping the 3'-end is another commonly used approach to block 3'-5' exonuclease attack. For example, an aptamer targeting SARS-CoV helicase was modified with 3'-biotin or 3'-inverted thymidine and showed strong resistance to nuclease attack in serum [57]. Such stable aptamers can be potentially used for clinical trials of drugs against SARS-CoV-2.

Recent progress in aptamer technology raises exciting opportunities for the detection and treatment of various microorganisms. Coupled with other cutting-edge molecular diagnostic and therapeutic methods, we believe that aptamers will also play a crucial role in the detection and inhibition of SARS-CoV-2.

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Declaration of interests

The authors declare no conflicts of interest.

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