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# Repurposing of SARS-CoV nucleocapsid protein specific nuclease resistant RNA aptamer for therapeutics against SARS-CoV-2

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## ABSTRACT

COVID-19 pandemic is rapidly advancing among human population. Development of new interventions including therapeutics and vaccines against SARS-CoV-2 will require time and validation before it could be made available for public use. Keeping in view of the emergent and evolving situation the motive is to repurpose and test the immediate efficacy of available drugs and therapeutics against COVID-19. Through this article we propose and discuss the possibility of repurposing the available nuclease resistant RNA aptamer against the nucleocapsid protein of SARS-CoV as a potential therapeutic agent for COVID-19.

## 1. Introduction

The world is currently facing a pandemic called COVID-19. This pandemic has spread across 205 countries and territories infecting more than 9 million individuals and causing death of more than 400,000 people (COVID-19 Coronavirus Pandemic, 2020). The pathogen responsible for COVID-19 is Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) belonging to *Beta-Coronaviridae* family. The symptoms of COVID-19 range from mild to severe, and include mainly fever, cough, and respiratory distress with severe cases progressing to pneumonia, hypoxemia and death (Chakrabarti et al., 2020). Considering the emergent situation, there is a need for rapid development of theranostics to control the outbreak of COVID-19. Though qRT PCR and antibody based diagnosis of SARS-CoV-2 are available and being utilized, there is no specific therapeutic agent or vaccine available till date. Thus, a major motive around the world is to repurpose the existing antivirals (remdesivir) and drugs (hydroxychloroquine and camostat mesylate) to test their immediate efficacy in controlling and alleviating the clinical symptoms and spread of COVID-19.

To support the initiative of repurposing and to fast track the development of probable interventions in COVID-19, we would like to propose the use of existing aptamers originally developed against SARS-CoV. Aptamers are small stretches of oligonucleotides (both DNA and RNA) of 10–100 nucleotides in length with excellent target binding specificity, cell internalization potential and no immunogenicity, suggestive of their probable direct applications under an emergent situation (Zou et al., 2019). It has been previously shown that anti-gp120 RNA aptamer-siRNA molecule successfully inhibited HIV-1 replication and reduced viral load both *in vitro* and *in vivo* (Zhou et al., 2013). During literature review, to search for probable aptamer candidates previously developed against SARS-CoV protein targets, we identified DNA and nuclease resistant RNA aptamers against nucleocapsid (N) protein of SARS-CoV (Table 1) (Cho et al., 2011; Ahn et al., 2009). However, no aptamer was retrieved for spike (S) protein. Both the nucleic acid aptamers have reportedly been evaluated for their diagnostic potential against the N protein of SARS-CoV, but their therapeutic utility has not been examined.

It has been reported that N protein is among the most conserved

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**Table 1**  
Available nucleic acid aptamers reported against nucleocapsid protein of SARS-CoV.

	Type	Aptamer Sequences	Dissociation Constant	Reference
Group 1	DNA	GGATGCGGAAACTGGCTAATTGGTGAGGCTGGGGCGGTCTGTGCA	4.93 ± 0.30 nM	Cho et al., 2011
		ACACGTGCGGAATCGTGGCTTGGTGGGTGTCGCGCCGGCGGATA	–	
		GGGCGCGGAGAACGCCGTGCTTGTCCGGGGCGCCGGTACTG	–	
		GCAGCCGTAAGGGTCTTGGGTGTTAGAGCCGCGCGGGCCCCCG	–	
Group 2	DNA	AATCGCTTGCTCTTGAGCTGGCAGTTCGTAGGCGGTGGGG	–	–
		AGAGCCCGTTTCTTGCCAGTTCGATGCACCTGGAGTGGG	–	
Group 3	DNA	CGATTGCAATACGAGAGTTTTCTCGGATCGTAAGCAAATTCA	–	–
		CGACAATAAGCTCATAGAGATATAAGTCCCAGCGAACTAAGGCTA	–	
Group 4	DNA	ACGGGCGAACTACGAATATATTCTCCTGTGCAGGCTCGTTGTG	–	–
		AATTATGGACAAGGAAAAATTCTAGGCCTCACACTATGGTCAGTG	–	
Aptamer 11	DNA	CCGTAGATCGAGGGAGCGCATTAAAGGTATACGCCCTTCCCATCTT	9.02 ± 1.89 nM	Ahn et al., 2009
Aptamer 1	RNA	UGUCGUUCGUGUCUUGCUACGUUACGUUACCGGUUGG	1.65 ± 0.41 nM	
Aptamer 2	RNA	UCAUACACACAUCUCACGGGAGACAUAGCUGACGAUAC	–	

proteins across the four coronaviridae families (McBride et al., 2014). Furthermore, N protein contains a highly positively charged region in its C-terminal dimerization domain and possesses strong affinity towards ssDNA, ssRNA and ds DNA (McBride et al., 2014). The N protein may also play an important role in promoting the SARS-CoV-2 replication by modulating the host cell responses such as by inhibition of RNAi machinery (Cui et al., 2015). Considering the conserved nature of N protein sequences between SARS-CoV and SARS-CoV-2 [Fig. 1A], and the affinity of the protein towards nucleic acids, we presumed that the previously designed nuclease resistant aptamers against N protein of SARS-CoV could be beneficial as therapeutic agents for COVID-19. Criteria for selecting and focusing the RNA aptamers included experimental validation of target binding sites in N protein, stability, dissociation constant and ribonucleotide composition of transfection motifs. To this effect, we analyzed and searched for the presence of common RNA motifs required for cell internalization in truncated RNA aptamer sequences (Magalhães et al., 2012). The detailed study carried out by Magalhães et al., 2012 on identifying a general RNA motif for cellular transfection provided an internalization signal which can be adapted for use as a general purpose reagent for transfection into a wide variety of cell types including primary cells. Furthermore, the findings and utility of the signal were also validated in C57BL/6 mice. The authors suggested two motifs, addition of which leads to generation of specific stem loop structure which is required for cell internalization. We further modified the two reported truncated RNA aptamer sequences by addition of common RNA motifs required for cell internalization at both 5' and 3' ends to create a molecule which may be fully capable of cell internalization. Interestingly, the modified RNA aptamer 1 sequence was able to retain its native stem loop structure both for transfection motifs and N-binding domain as shown by structure analysis done using Mfold indicating the retention of target binding capability [Fig. 1 B-D] (Zuker, 2003). However, in case of RNA aptamer

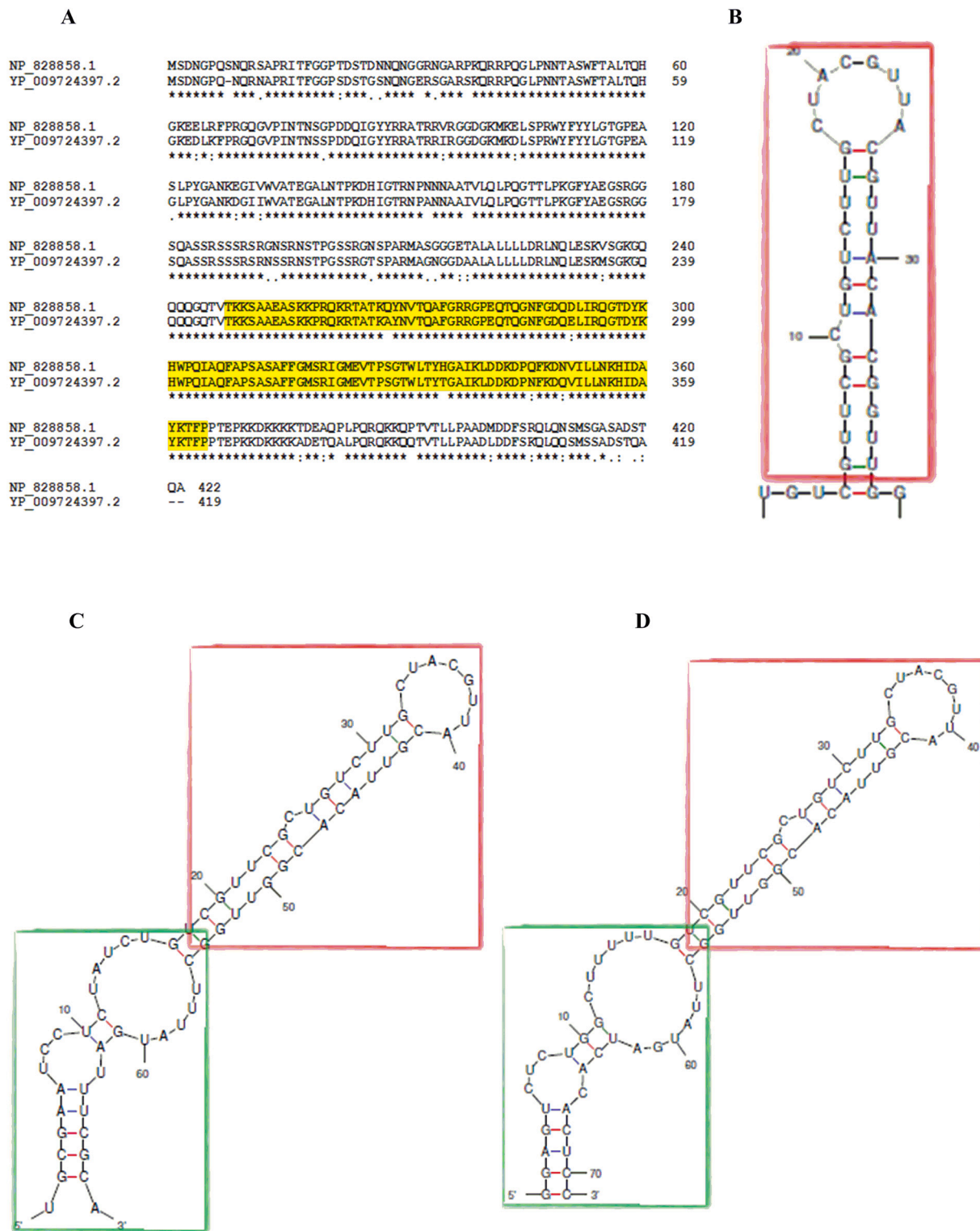
2 the conformation of structure was altered suggesting a possible loss of specific target binding capacity.

The nuclease resistant RNA aptamer 1 with probable cell internalization potential may thus be utilized to target N-protein and thereby interfere with viral replication. Additionally, RNA aptamer-siRNA chimeric molecule may be designed for targeting the RNA genome/transcript of SARS-CoV-2.

Moreover, considering S protein, no aptamer molecule was reported thus impeding the possibility of repurposing until recently. Song et al. have developed new aptamers targeting S protein of SARS-CoV-2 and particularly receptor binding domain (RBD). The authors reported  $K_d$  values of 5.8 and 19.9 nM for CoV2-RBD-1C and CoV2-RBD-4C DNA aptamers respectively (Song et al., 2020). The aptamers needs to be further evaluated for their ability to interfere and disrupt binding between host ACE2 and RBD of spike protein both *in vitro* and *in vivo*.

Spike protein is a large membrane protein and may be targeted for prevention of virus internalization into host cell and neutralization of viral molecules by newly identified DNA aptamers. Another important aspect is that pre- and post fusion conformation changes acquired by spike protein may change its recognition sites utilized by currently identified novel aptamers thus limiting their direct potential post-internalization. However they can potentially be evaluated as a carrier molecule for delivering small RNA targeting the viral transcripts in line with our proposed approach subject to retention of native structure upon motif addition.

In conclusion, it is better to go for selection of novel aptamers against new targets, however considering the emergent situation, conserved profile of N protein, affinity of N protein towards nucleic acids, modular and customizable properties of aptamers allow for considering repurposing and may yield a therapeutic molecule which could prove to be advantageous and beneficial against SARS-CoV-2.



**Fig. 1.** (A): Sequence alignment of nucleocapsid protein from SARS-CoV (Accession: NP\_828858.1) and SARS-CoV-2 (Accession: YP\_009724397.2). Yellow highlighted region represents conserved dimerization domain in nucleocapsid protein. (B-D): Sequences and secondary structure of nuclease resistant RNA aptamers as analyzed using Mfold program. B: Truncated RNA aptamer 1 consisting of probable N binding stem loop domain. C, D: Two different structures of modified truncated RNA aptamer 1 consisting of 5' pyrimidine rich region and 3' consensus motif for cell internalization. Red Box: Probable N-binding domain. Green Box: RNA motifs required for cell internalization.

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## Declaration of Competing Interest

The author(s) declare that they have no competing interests.

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