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## Review article

# The nucleocapsid protein of zoonotic betacoronaviruses is an attractive target for antiviral drug discovery

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## ABSTRACT

Betacoronaviruses are in one genera of coronaviruses including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome-related coronavirus (MERS-CoV), etc. These viruses threaten public health and cause dramatic economic losses. The nucleocapsid (N) protein is a structural protein of betacoronaviruses with multiple functions such as forming viral capsids with viral RNA, interacting with viral membrane protein to form the virus core with RNA, binding to several cellular kinases for signal transductions, etc. In this review, we highlighted the potential of the N protein as a suitable antiviral target from different perspectives, including structure, functions, and antiviral strategies for combatting betacoronaviruses.

## 1. Introduction

There are four genera of coronaviruses (CoVs) in the subfamily *Orthocoronavirinae* of *Coronaviridae* family: alphacoronaviruses ( $\alpha$ -CoVs), betacoronaviruses ( $\beta$ -CoVs), gammacoronaviruses ( $\gamma$ -CoVs), and deltacoronaviruses ( $\delta$ -CoVs) [1]. CoVs have been identified in different mammals and fowl such as dogs, cats, horses, bats, cattle, swine, mice, whales, monkeys, ferrets, camels, turkeys, and chickens [2]. In humans, zoonotic-origin  $\beta$ -CoVs are of the greatest importance. The human coronaviruses (HCoVs), including HCoV-HKU1 and HCoV-OC43, are responsible for 10%–20% of common colds [2]. SARS-CoV caused the 2002–2003 severe acute respiratory syndrome (SARS) pandemic; Middle East respiratory syndrome (MERS)-CoV led to the 2012 MERS epidemic in the Middle East; and SARS-CoV-2 resulted in the coronavirus disease 2019 (COVID-19) pandemic. Diseases related to  $\beta$ -CoVs in humans range from asymptomatic to respiratory infections, enteric infections, encephalitis, and the worst outcome, death [3–8]. Currently, there are four lineages within the genus *Betacoronavirus*: lineage A (HCoV-OC43, HCoV-HKU1, etc.), lineage B (SARS-CoV, SARS-CoV-2, etc.), lineage C (MERS-CoV, *Tylonycteris* bat coronavirus HKU4 (BtCoV-HKU4), etc.), and lineage D (Rousettus bat coronavirus HKU9 (BtCoV-HKU9), etc.) [9]. In this review, we will mainly focus on the antiviral aspects of the N protein in zoonotic  $\beta$ -CoVs.

CoVs infect host cells primarily by receptor binding via the viral

spike (S) protein, which mediates viral and host cell membrane fusion triggered by conformational changes of the S protein. After entering the cytoplasm and uncoating, the virus releases the nucleocapsid and the viral genome, followed by initiation of viral replication and transcription in cytoplasm. As the largest known RNA virus, CoVs have a positive-sense, single-stranded RNA with a genome size ranging from 26 to 32 kb packaged in the enveloped virion [10]. Two-thirds of the genome is the 5'.

Non-structural protein (NSP) coding regions encoding two overlapping viral replicase proteins, polyproteins 1a (pp1a) and pp1ab [11]. The polyproteins are cleaved into mature NSPs that are related to RNA synthesis by virally encoded papain-like (PL) and 3 chymotrypsin-like (3CL) proteases [12–14]. The last third of the genome encodes structural (SP), envelope (E), membrane (M), and nucleocapsid (N) proteins and nonessential accessory proteins by means of producing subgenomic (sg) mRNAs [11,15]. The replicated RNA genome forms a nucleocapsid with the N protein and is packaged into an unmaturing virion. The virion is matured in the Golgi and transported in exocytic vesicles. After plasma membrane fusion, the mature virus is released from the infected cell [16].

2. Structure of the  $\beta$ -CoV N protein

The N protein structure is conserved within different members of

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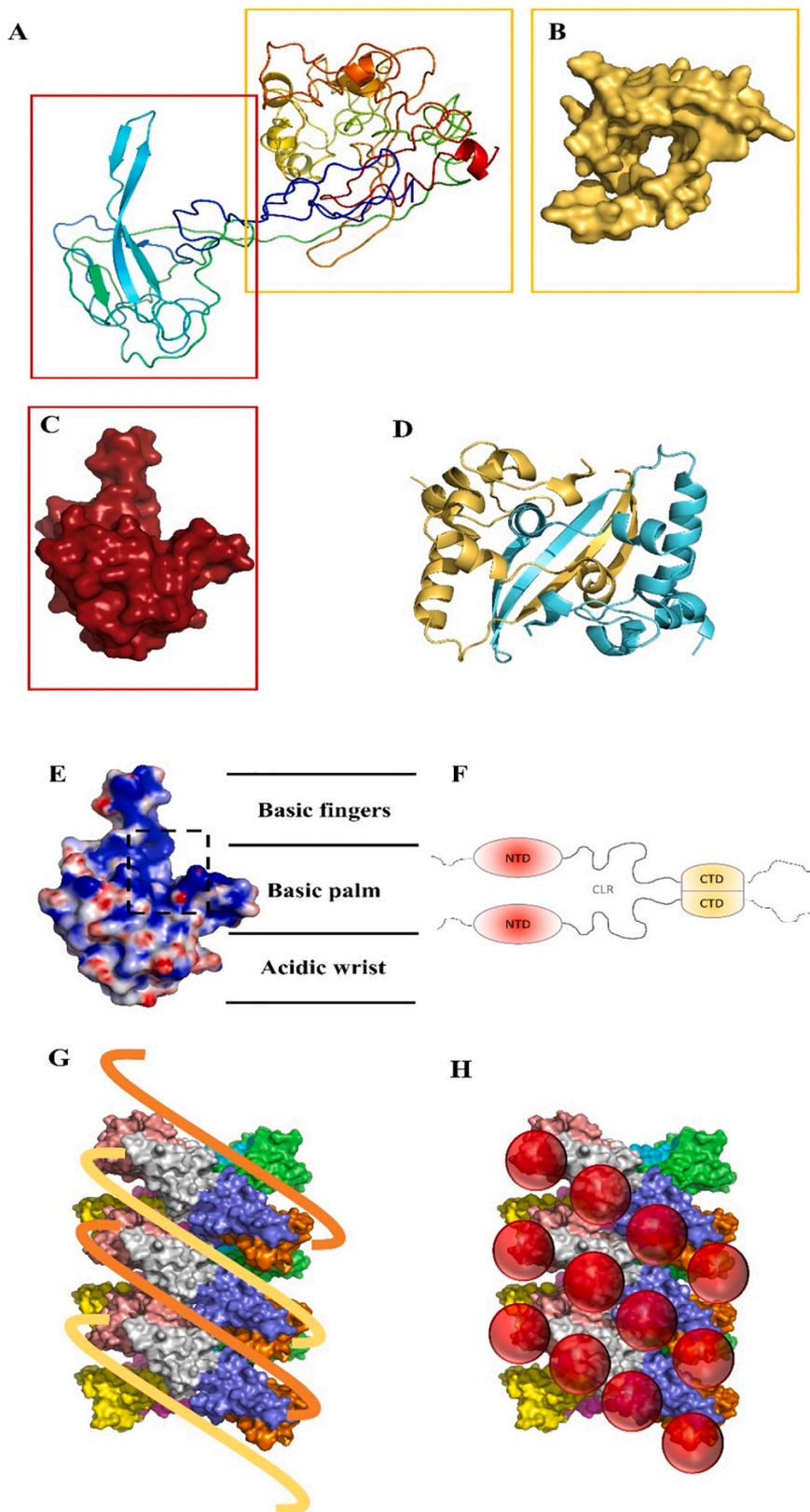
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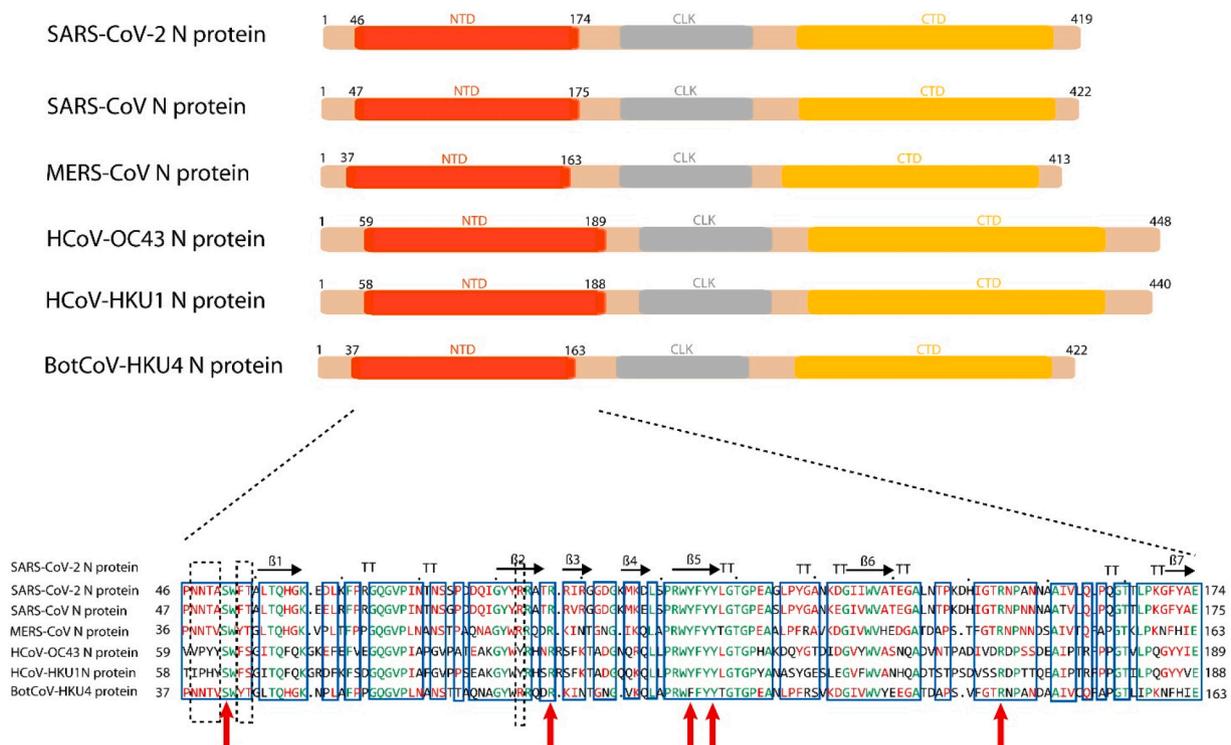
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**Fig. 1.** Structure of the  $\beta$ -CoV N protein. (A) C-I-TASSER structure model. The ribbon representations of the structures of NTD (N-terminal RNA-binding domain, red box) and CTD (C-terminal dimerization domain, yellow box) are generated with PyMOL from coordinates in the protein data bank (PDB IDs: NTD, 6M3M; CTD, 6WJI). The relative orientation of NTD, CTD, and CLR are drawn randomly to reflect the dynamic nature of the N protein. (B) CTD. (C) NTD. (D) CTD dimer. (E) Electrostatic surface of the NTD. Blue denotes positive charge potential. Red denotes negative charge potential. Pocket in the square indicates the RNA binding site of NTD; this pocket is various among  $\beta$ -CoVs. (F) The domain organization of the  $\beta$ -CoV N protein. (G) The crystal packing of the CTD 24-mer. Yellow and orange ribbons represent  $\beta$ -CoV viral RNA strands wrapping around the helical oligomer structure. (H) Schematic of the docking of NTD onto the CTD 24-mer complex. The NTD domains are represented by red ellipsoids. Structures were generated using PyMOL (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

$\beta$ -CoVs. Amino acid (aa) sequence comparisons revealed three distinct and highly conserved domains: a hand-shaped N terminal domain (NTD) (Fig. 1C); a dimerization-related C-terminal domain (CTD) (Fig. 1B); and the disordered central linker region (RNA-binding domain, CLR) (Fig. 1A) [10,14,17]. All three domains are related to RNA binding

[18,19]. Because of the characteristics of the N protein, such as poor stability and dynamic behavior, no crystal structures were solved for full-length N proteins of coronaviruses. In solution, the full-length SARS-CoV N protein predominantly exists as a CTD dimer that is considered to be the basic building blocks of the nucleocapsid (Fig. 1D) [20–22]. A



**Fig. 2.** Domain architectures of  $\beta$ -CoVs N protein. NTD: N-terminal RNA-binding domain; CTD: C-terminal dimerization domain. Multiple sequence alignment of SARS-CoV-2 N-NTD (GenBank: NC\_045512.2) with SARS-CoV N-NTD (GenBank: NC\_004718.3), MERS-CoV N-NTD (GenBank: NC\_019843.3), HCoV-OC43 N-NTD (GenBank: NC\_006213.1), HCoV-HKU1 (GenBank: NC\_006577.2), Tylonycteris bat coronavirus HKU4 (GenBank: MH002339.1). Red arrows indicate conserved residues for ribonucleotide binding sites, dash-bordered boxes indicate variability of residues in the structural comparisons. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

structural model for a di-domain (DD) has been proposed by fitting small angle X-ray scattering data for the SARS-CoV N protein [23]. The CTD dimer forms as a core with NTDs branching out as two arms connecting to the core via CLR (Fig. 1F). As NTD and CTD are independently folded structural regions, research groups usually study them separately.

Although NTDs are divergent in both sequence and length within *Coronaviridae* [24], they are relatively conserved within *Betacoronavirus* genera. As shown in Fig. 2, NTD has been mapped for HCoV-OC43 (aa 58–195) [25], SARS-CoV-2 (aa 46–174) [26], SARS-CoV (aa 45–181) [27], and MERS-CoV (aa 39–165) [28]. NTD within zoonotic  $\beta$ -CoVs displays a right-handed (loops)-( $\beta$ -sheet core)-(loops) sandwiched structure which is conserved among all NTD in CoVs (Fig. 2) [26,29]. The hand-shaped NTD is represented by basic fingers, a hydrophobic basic palm, and an acidic wrist (Fig. 1E) [26,30]. The hand-shaped NTD residues in the middle part are more conserved than residues located in the basic fingers and acidic wrist [26]. Based on the crystal structures of  $\beta$ -CoVs-NTD [26,28,31,32], critical residues have been identified related to RNA binding in  $\beta$ -CoVs-NTD. The pockets of NTDs of SARS-CoV-2 and SARS-CoV are distinct from those of HCoV-OC43 NTD. For HCoV-OC43, the co-crystal structure of HCoV-OC43 NTD with adenosine monophosphate (AMP) revealed the AMP binding site composed of residues Ser 64, Gly 68, Arg 122, Tyr 124, Tyr 126, and Arg 164 [33].

The NTD and CTD are linked by CLR, which is rich in serine and arginine residues. The CLR also contains abundant of phosphorylation sites which facilitate the N protein to become involved in cell signaling [34–38]. The flexibility of CLR facilitates its direct interactions between the N protein and RNAs [23,27,39–41]. However, because there is no structural information for the CLR, and protein phosphorylation is reversible, opposing hypotheses on the function of CLR in N protein oligomerization have been proposed based on different facts. Because phosphorylation can stabilize the N protein by reducing the total positive charge, hyperphosphorylation of CLR could enhance and regulate

oligomerization of DD [42]. In another study, it was reported that oligomerization might be impaired when the CLR is phosphorylated [43,44]. These results suggest that phosphorylation may act as a key to lock or unlock N protein oligomerization.

Similar to that of NTD, sequence and length of the CTD are relatively conserved within the  $\beta$ -CoV genera, indicating similar structural and functional roles for the CTD [45]. The monomer of the SARS-CoV N protein is unstable because the CTD folds into an extended conformation with a topology of  $\alpha 1$ - $\alpha 2$ - $\alpha 3$ - $\alpha 4$ - $\alpha 5$ - $\alpha 6$ - $\beta 1$ - $\beta 2$ - $\alpha 7$ - $\alpha 8$ , leading to a large cavity in its center [45,46]. The CTD can be stabilized through domain-swapped dimerization by inserting a  $\beta$ -hairpin of one subunit into the center cavity of the opposite subunit [21,22,45,46,47,48]. Exploration of the crystal structure of SARS CTD revealed a CTD octamer formed by two butterfly-shaped tetramers responsible for RNA-binding activity in the CTD [46]. The dimerization core has been identified at aa 281–365. A positively charged groove related to RNA binding has been mapped to aa 248–280 that contained eight positively charged lysine and arginine residues [46,48]. Based on the crystal structure, a helical supercomplex structure is formed by these octamers and is characterized by a continuous positively charged surface [46]. Through electrostatic interactions between the continuous positively charged surface of the CTD supercomplex and negatively charged RNA strands, the viral RNA strands can bind and wrap around the CTD supercomplex by non-specific charge interactions (Fig. 1G, H) [46,49]. Similar to human CoV 229E (hCoV-229E), all zoonotic  $\beta$ -CoV NPs have C-terminal tail peptides. In hCoV-229E, the disordered C-terminal tail is responsible for dimer-dimer association. It has been reported that peptide N377–389 from the C-terminus of hCoV-229E has an inhibitory effect on viral titer of HCoV-229E [47]. Understanding the mechanism of how the C-terminal tail peptide in the  $\beta$ -CoV N protein is involved in oligomerization may shed light on identifying antiviral targets for drug discovery to combat  $\beta$ -CoVs by means of disrupting the N protein self-association.

### 3. Functions of the $\beta$ -CoV N protein

The primary function of the  $\beta$ -CoV N protein is to package the viral genomic RNA into nucleocapsids. This structure not only protects the genome, but also further guarantees that the replication and transmission can be done in a timely and reliable manner. Therefore, a correct and stable structure is important for nucleocapsid function. The  $\beta$ -CoV N protein uses its dimer form as a basic building block for nucleocapsid formation [30]. Based on the crystal structure of SARS-CoV CTD, the dimerization core has been identified at aa 281–365 [46].

The N protein, M protein, and gRNA form the internal spherical/icosahedral core of CoVs [50,51]. Although the M protein can form virus-like particle (VLP) alone via self-assembly, both particle densities and uniformity are lower than VLPs formed by M protein + N protein [50]. This suggests that the incorporation of N into M vesicles stabilizes the formation of VLPs. For SARS-CoV, aa 168–208 in the N protein and a highly polar and hydrophilic region (aa 194–205) in the M protein are responsible for the interaction via an ionic interaction [52–54]. He et al. demonstrated that serine/arginine-rich motif (SSRSSRSRGNSR) in CLR at aa 184–196 is also critical for multimerization of the N protein [55]. Because the same motif is involved in both N-N and N-M interactions, this region may be critical for maintaining correct conformation of the N protein. Because the sequence homology of N and M proteins among zoonotic  $\beta$ -CoVs is relatively conserved, N-M and N-N protein interactions of SARS-CoV can be representative [56]. Similar N-M interaction has been observed in mouse hepatitis virus (MHV) and transmissible gastroenteritis virus (TGEV) [51,57,58], suggesting that the CoVs employ the same method to facilitate N-M protein interaction, although the sequence homology of N and M proteins among them is low [59,60].

During the early stage of virus replication cycle, the N protein accumulates in cytoplasm with viral gRNA and nonstructural protein 3 (nsp3), suggesting the N protein might play a critical role in viral transcription and translation [61–63]. In MHV, a commonly used model of  $\beta$ -CoVs, both CLR and NTD are involved, interacting directly with nsp3 as part of the replication-transcription complexes (RTCs) related to CoV RNA synthesis [64,65]. The binding between nsp3 and CLR in MHV may induce a conformational change at the CLR of the N protein, subsequently regulating the intracellular localization of the N protein [66] and/or other RNA binding functions. In addition, the reverse genetics to rescue SARS-CoV indicates the N protein might play a critical role in enhancing the translation of viral mRNAs or enhancing subgenomic transcription [67].

The N protein can be detected in both cytoplasm and nucleolus for different CoVs, suggesting nucleolar localization of the N protein is a shared feature within the coronavirus family and is possibly of functional significance [35,68–71]. For the SARS-CoV N protein, the nuclear export signal motif identified at aa 324-EVTPSGTWLT-334 (CTD) is the dominant signal in determining N protein localization [72]. Phosphorylated N protein can be translocated to the cytoplasm from the nucleus by binding to the 14-3-3 protein [43,73], although the phosphorylation of the N protein happens in both sites [20]. Moreover, the absence of SR-rich domain at the CLR of the SARS-CoV N protein can dramatically change localization of the N protein [55]. The functions of the N protein in nucleolus still remain elusive.

Through interaction with host components, the N protein can inhibit protein translation via EF1 $\alpha$ -mediated action [70], modulate the host cell cycle via cyclin-CDK activity regulation [73], induce apoptosis [74], cause lung inflammation in SARS-CoV infected patients via activation of cyclooxygenase-2 (COX-2) [75], and inhibit the synthesis of interferons [76,77].

### 4. Antiviral strategies for $\beta$ -CoVs

Since the 1960s, antivirals targeting many different viral diseases have been identified based on different mechanisms [78–80].

**Table 1**  
 $\beta$ -CoV antiviral strategies.

	Targets	Example inhibitors
Virus-directed strategies	Viral structural proteins	Chloroquine, nafamostat, griffithsin
	Viral helicase	Bananins, adamantanes
	Viral protease	3C-like protease inhibitors: cinanserin Papain-like protease inhibitors: disulfiram
Host-directed strategies	RdRp	Ribavirin, remdesivir, favipiravir
	ACE2	Human recombinant soluble ACE2
	Interferons	Interferon $\alpha$ , interferon $\beta$ , interferon $\gamma$

Approximately 90 compounds have been formally licensed to use as clinical antiviral therapies; half of these are used for the treatment of human immunodeficiency virus (HIV) infection [81,82]. Other approved antiviral drugs are designed for hepadnavirus, hepatitis virus, herpes simplex virus, influenza viruses, human cytomegalovirus, varicella-zoster virus, respiratory syncytial virus, and human papillomavirus [82]. However, because of specific licensing, antiviral therapies are still unavailable for many critical emerging viral diseases.

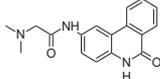
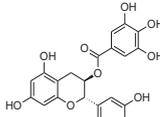
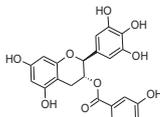
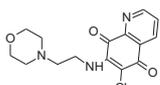
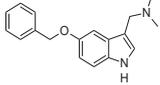
Ribavirin, a nucleoside analogue similar to remdesivir, can inhibit a broad spectrum of viruses in vitro and in vivo with pleiotropic mechanisms including inhibition of viral capping enzymes, lethal mutagenesis of viral RNA genomes, and inhibition of viral RNA synthesis [83,84]. However, a single aa change in the viral RdRp can result in resistance in poliovirus; although a 99.3% loss in infectivity was observed after ribavirin treatment, it caused a 9.7-fold increase in mutagenesis [85,86]. The mutant RdRp has a greater fidelity compared with that of the parental strain.

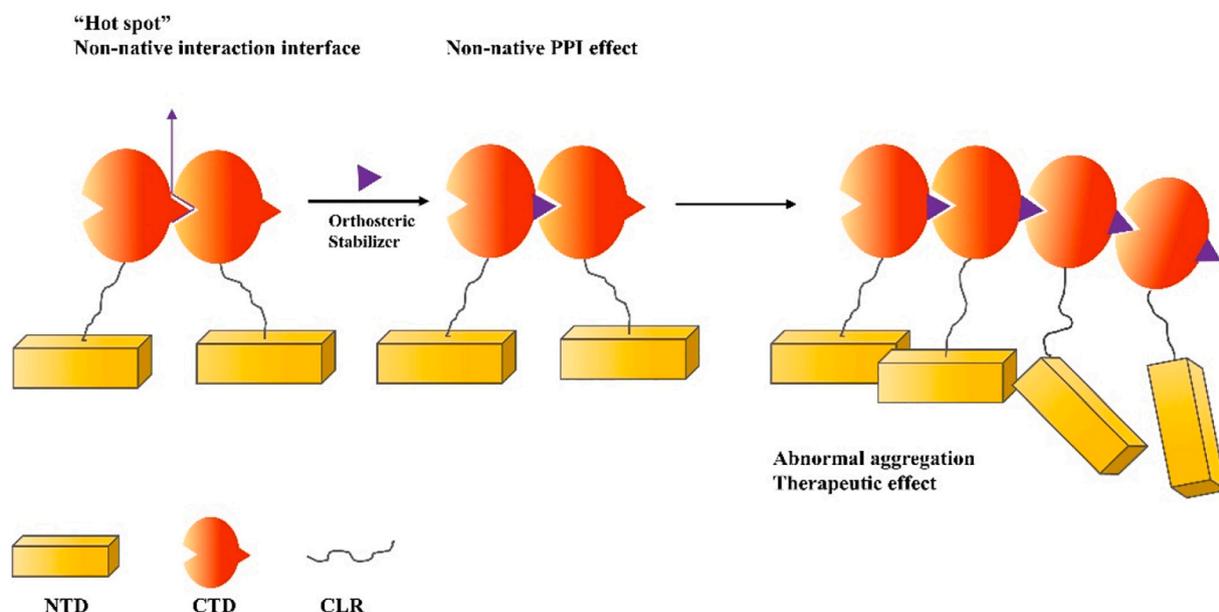
Neuraminidase inhibitors (NAIs) are a group of antiviral therapeutics to treat infection caused by influenza A and B viruses. The primary function of NA, a surface protein of influenza viruses, is to cleave sialic acids from the infected cell surface and subsequently release the newly formed mature viruses. After identifying of the mechanism of viral release of influenza, scientists developed NAIs that mimic the structure of sialic acids. After NAI treatment, the mature influenza viruses cannot be released to spread further because of inhibition of NA by NAI [87]. Unfortunately, due to the chronic use of NAIs, several NAI-resistant mutations have been identified in different strains all over the world [88–91]. Although  $\beta$ -CoVs have a relatively low mutation rate due to their proofreading machinery [92], they can still gain resistance to a specific antiviral treatment by mutating over time, which could be a potential risk to public health. To avoid generating directed resistant mutations caused by a single type of treatment, a combination of antiviral therapeutics with different mechanisms should be employed to treat the viral infection. As more than nine million life-years were saved from HIV infection by combination antiretroviral therapy, we should identify multiple antivirals to create a similar therapeutic method to control other important pathogens such as zoonotic  $\beta$ -CoVs [93].

Currently, there is no licensed antiviral drug approved for treatment of zoonotic  $\beta$ -CoV infections in the U.S., although the investigational nucleotide analogue remdesivir, an inhibitor of RNA-dependent RNA polymerase (RdRp) in SARS-CoV-2, is in clinical trial program [94]. However, we need to carefully consider putting these drugs into human clinical trials. In addition to potential side effects, antiviral drugs may worsen the situation if drug-resistant strains emerge. Also, unlike most antibiotics, specific antivirals are designed to target specific viral proteins that may be involved in normal human functions instead of simply inactivating the pathogen [95].

As shown in Table 1, there are two main perspectives for therapy development against  $\beta$ -CoVs based on previous experience: 1, virus-directed; 2, host-directed [96]. Because of the pandemic, numerous studies using have been carrying out worldwide and clinical trials are

**Table 2**  
β-CoV inhibitors target N proteins.

Full name	Compound abbreviations	Structure	Target site	Mechanism	Reference
N-(6-oxo-5,6-dihydrophenanthridin-2-yl)(N,N-dimethylamino)acetamide hydrochloride	PJ34		NTD	Inhibit RNA binding	[33]
(-)-Catechin gallate			NTD	Inhibit RNA binding	[97]
(-)-Gallocatechin gallate			NTD	Inhibit RNA binding	[97]
6-Chloro-7-(2-morpholin-4-yl-ethylamino) quinoxaline-5,8-dione	H3		NTD	Inhibit RNA binding	[98]
5-Benzyloxygramine	P3		NTD	Induced abnormal dimerization	[100]



**Fig. 3.** Schematic describing the rationale used in designing the allosteric stabilizer of this study. An orthosteric stabilizer is used to bind to the non-native interaction interface of the NTD and stabilize the abnormal interaction between proteins. Then the CTD cannot be packed correctly.

updating on [ClinicalTrials.gov](https://www.clinicaltrials.gov) almost every day. Numerous strategies have been explored and reviewed previously [96]. In this review, we focus on antiviral strategies targeting the viral N protein.

Since the N protein of β-CoVs is a multifunctional structural protein with conserved structure, it is an attractive target for discovery of antiviral drugs. Several groups are developing antiviral drugs targeting N protein. Roh, Changyun found (-)-catechin gallate and (-)-gallocatechin gallate have a remarkable inhibition activity on SARS-CoV N protein by quantum dots-conjugated RNA oligonucleotide on a biochip platform [97].

The compound N-(6-oxo-5,6-dihydrophenanthridin-2-yl)(N,N-dimethylamino)acetamide hydrochloride (PJ34) was identified as an N protein inhibitor which can reduce the N protein's RNA-binding affinity,

leading to inhibition of viral replication at 10 μM (Table 2) [33]. Subsequently, they found that 6-chloro-7-(2-morpholin-4-yl-ethylamino) quinoxaline-5,8-dione (small-compound H3) worked as an RNA binding inhibitor against HCoV-OC43 by targeting NTD, which can significantly reduce the RNA-binding capacity of N protein [98]. For SARS-CoV, nuclear magnetic resonance (NMR) was employed to screen small molecules that bind to the NTD of SARS CoV with low affinity (1 mM) [99]. A compound 6-amino-4-hydroxy-naphthalene-2-sulfonic acid was identified as a potent candidate to bind to the same pocket site of NTD as RNA. For SARS-CoV-2, Kang et al. identified a hydrophobic pocket consisting of Phe 57, Pro 61, Tyr 63, Tyr 102, Tyr 124, and Tyr 126 for SARS-CoV-2 [26]. Therefore, this pocket might be a potential drug targeting site for drug screening, and the same group is

doing further research to confirm their hypothesis.

In recent research on the MERS-CoV N protein, an antiviral drug design yielded structure-based stabilization of non-native protein-protein interactions at NTD, leading to an abnormal N protein oligomerization (Fig. 3) [100]. The structure of NTD revealed a conserved hydrophobic pocket consisting of W43, N66, N68, Y102, and F135 of one monomer at the dimerization interface, which accommodates the side chain of M38 of the second monomer. Subsequently, virtual screening by targeting the hydrophobic pocket of the NTD dimeric interface identified 5-benzyloxygramine (P3) as a candidate binder. In cell culture, P3 can significantly suppress N protein expression at 100  $\mu\text{M}$  [100]. Although discovery of antiviral drugs usually involves native protein-protein interactions (PPIs), identification and stabilization of non-native PPIs could also be taken into consideration for drug discovery to counter CoV infections.

In addition, because only high-biosafety-level labs can handle the virulent strains of zoonotic  $\beta$ -CoVs, hindering the speed of antiviral discovery, it is crucial to find suitable models to study the antivirals in lower-biosafety-level labs to initiate the primary screening of antiviral candidates. VLPs without infectious properties should be functional and safe models for this purpose [50]. For example, based on N protein functions, we know it mainly serves as a structural protein. So, if the conformation of the N protein changes, VLPs might not be able to process successfully [101,102]. Thus, we can use VLPs to screen antiviral candidates at the cell base level.

## 5. Conclusions

Currently, we have an urgent unmet medical need for antiviral drugs with different mechanisms to combat  $\beta$ -CoV pandemics or epidemics. The N protein of  $\beta$ -CoVs is an attractive and suitable therapeutic target for the following reasons: first, the N protein has many conserved sites related to its structural characteristics; second, the N protein has multiple functions, some of which are critical for virus replication; and third, we can use VLPs as a tool to initiate the primary screening of antiviral candidates in lower-biosafety-level labs.

## Declaration of competing interest

The authors declare no competing financial interest.

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## References

- [1] The International Committee for Taxonomy of Viruses (ICTV) [(accessed on 27 June 2014)]. Available online: [http://talk.ictvonline.org/files/ictv\\_documents/m/msl/4090.aspx](http://talk.ictvonline.org/files/ictv_documents/m/msl/4090.aspx).
- [2] Luis Enjuanes (Ed.), *Coronavirus Replication and Reverse Genetics* vol. 287, Springer Science & Business Media, 2004.
- [3] Takeshi Moriguchi, et al., A first case of meningitis/encephalitis associated with SARS-Coronavirus-2, *Int. J. Infect. Dis.* 94 (2020) 55–58.
- [4] Jinyang Gu, Bing Han, Jian Wang, COVID-19: gastrointestinal manifestations and potential fecal-oral transmission, *Gastroenterology* 158 (6) (2020) 1518–1519.
- [5] Astrid Vabret, et al., An outbreak of coronavirus OC43 respiratory infection in Normandy, France, *Clin. Infect. Dis.* 36 (8) (2003) 985–989.
- [6] Zhiliang Hu, et al., Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China, *Sci. China Life Sci.* (2020) 1–6.
- [7] R.L. Graham, E.F. Donaldson, R.S. Baric, A decade after SARS: strategies for controlling emerging coronaviruses, *Nat. Rev. Microbiol.* 11 (2013) 836–848, <https://doi.org/10.1038/nrmicro3143>.
- [8] B.C. Fielding, Human coronavirus NL63: a clinically important virus? *Future Microbiol.* 6 (2011) 153–159, <https://doi.org/10.2217/fmb.10.166>.
- [9] A.C.P. Wong, X. Li, S.K.P. Lau, P.C.Y. Woo, Global epidemiology of bat coronaviruses, *Viruses* 11 (2) (2019) 174. Published 2019 Feb 20, <https://doi.org/10.3390/v11020174>.
- [10] M.M. Lai, D. Cavanagh, The molecular biology of coronaviruses, *Adv. Virus Res.* 48 (1997) 1–100.
- [11] Paul S. Masters, The molecular biology of coronaviruses, *Adv. Virus Res.* 66 (2006) 193–292.
- [12] Tomonari Muramatsu, et al., SARS-CoV 3CL protease cleaves its C-terminal autoprocessing site by novel subsite cooperativity, *Proc. Natl. Acad. Sci.* 113 (46) (2016) 12997–13002.
- [13] Min-Han Lin, et al., Structural and functional characterization of MERS coronavirus papain-like protease, *J. Biomed. Sci.* 21 (1) (2014) 54.
- [14] S. Perlman, J. Netland, Coronaviruses post-SARS: update on replication and pathogenesis, *Nat. Rev. Microbiol.* 7 (2009) 439–450, <https://doi.org/10.1038/nrmicro2147>.
- [15] E.J. Snijder, P.J. Bredenbeek, J.C. Dobbe, V. Thiel, J. Ziebuhr, L.L. Poon, Y. Guan, M. Rozanov, W.J. Spaan, A.E. Gorbalenya, Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage, *J. Mol. Biol.* 331 (2003) 991–1004, [https://doi.org/10.1016/S0022-2836\(03\)00865-9](https://doi.org/10.1016/S0022-2836(03)00865-9).
- [16] Michael M.C. Lai, *Coronaviridae: The Viruses and Their Replication*, 2001.
- [17] C.K. Chang, S.C. Lo, Y.S. Wang, M.H. Hou, Recent insights into the development of therapeutics against coronavirus diseases by targeting N protein, *Drug Discov. Today* 21 (2016) 562–572.
- [18] H. Laude, P. Masters, *Coronaviruses and arteriviruses*, in: *The Coronavirus Nucleocapsid Protein*, Plenum Press, New York, NY, USA, 1995, pp. 141–163.
- [19] C.A. de Haan, P.J.M. Rottier, Molecular interactions in the assembly of coronaviruses, *Adv. Virus Res.* 64 (2005) 165–230.
- [20] Chung-ke Chang, et al., The dimer interface of the SARS coronavirus nucleocapsid protein adapts a porcine respiratory and reproductive syndrome virus-like structure, *FEBS Lett.* 579 (25) (2005) 5663–5668.
- [21] Milan Surjit, et al., The nucleocapsid protein of the SARS coronavirus is capable of self-association through a C-terminal 209 amino acid interaction domain, *Biochem. Biophys. Res. Commun.* 317 (4) (2004) 1030–1036.
- [22] I-Mei Yu, et al., Recombinant severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein forms a dimer through its C-terminal domain, *J. Biol. Chem.* 280 (24) (2005) 23280–23286.
- [23] C.K. Chang, Y.L. Hsu, Y.H. Chang, F.A. Chao, M.C. Wu, Y.S. Huang, C.K. Hu, T. H. Huang, Multiple nucleic acid binding sites and intrinsic disorder of severe acute respiratory syndrome coronavirus nucleocapsid protein: implications for ribonucleocapsid protein packaging, *J. Virol.* 83 (2009) 2255–2264, <https://doi.org/10.1128/JVI.02001-08>.
- [24] R. McBride, M. van Zyl, B.C. Fielding, The coronavirus nucleocapsid is a multifunctional protein, *Viruses* 6 (8) (2014) 2991–3018. Published 2014 Aug 7, <https://doi.org/10.3390/v6082991>.
- [25] I.J. Chen, C.C. Chou, C.L. Liu, C.C. Lee, L.S. Kam, M.H. Hou, Crystallization and preliminary X-ray diffraction analysis of the N-terminal domain of human coronavirus OC43 nucleocapsid protein, *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* 66 (2010) 815–818.
- [26] Sisi Kang, et al., Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites, *Acta Pharm. Sin.* B 10 (2020) 1228–1238.
- [27] Chung-ke Chang, et al., Modular organization of SARS coronavirus nucleocapsid protein, *J. Biomed. Sci.* 13 (1) (2006) 59–72.
- [28] Y.-S. Wang, Chung-ke Chang, M.-H. Hou, Crystallographic analysis of the N-terminal domain of Middle East respiratory syndrome coronavirus nucleocapsid protein, *Acta Crystallographica Section F: Structural Biology Communications* 71 (8) (2015) 977–980.
- [29] H. Jayaram, H. Fan, B.R. Bowman, A. Ooi, J. Jayaram, E.W. Collisson, J. Lescar, B.V. Prasad, X-ray structures of the N- and C-terminal domains of a coronavirus nucleocapsid protein: implications for nucleocapsid formation, *J. Virol.* 80 (2006) 6612–6620, <https://doi.org/10.1128/JVI.00157-06>.
- [30] H. Fan, A. Ooi, Y.W. Tan, S. Wang, S. Fang, D.X. Liu, J. Lescar, The nucleocapsid protein of coronavirus infectious bronchitis virus: crystal structure of its N-terminal domain and multimerization properties, *Structure* 13 (2005) 1859–1868, <https://doi.org/10.1016/j.str.2005.08.021>.
- [31] I-Jung Chen, et al., Crystal structure-based exploration of the important role of Arg106 in the RNA-binding domain of human coronavirus OC43 nucleocapsid protein, *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* 1834 (6) (2013) 1054–1062.
- [32] Kumar Singh Saikatendu, et al., Ribonucleocapsid formation of severe acute respiratory syndrome coronavirus through molecular action of the N-terminal domain of N protein, *J. Virol.* 81 (8) (2007) 3913–3921.
- [33] Shing-Yen Lin, et al., Structural basis for the identification of the N-terminal domain of coronavirus nucleocapsid protein as an antiviral target, *J. Med. Chem.* 57 (6) (2014) 2247–2257.
- [34] M.M. Parker, P.S. Masters, Sequence comparison of the N genes of five strains of the coronavirus mouse hepatitis virus suggests a three domain structure for the nucleocapsid protein, *Virology* 179 (1990) 463–468, [https://doi.org/10.1016/0042-6822\(90\)90316-J](https://doi.org/10.1016/0042-6822(90)90316-J).
- [35] K.R. Hurst, C.A. Koetzner, P.S. Masters, Identification of *in vivo*-interacting domains of the murine coronavirus nucleocapsid protein, *J. Virol.* 83 (2009) 7221–7234, <https://doi.org/10.1128/JVI.00440-09>.
- [36] You J., Dove B.K., Enjuanes L., DeDiego M.L., Alvarez E., Howell G., Heinen P., Zambon M., Hiscox J.A. Subcellular localization of the severe acute respiratory syndrome coronavirus nucleocapsid protein. *J. Gen. Virol.* 2005;86 Pt 12: 3303–3310.

- [37] S.A. Stohlman, R.S. Baric, G.N. Nelson, L.H. Soe, L.M. Welter, R.J. Deans, Specific interaction between coronavirus leader RNA and nucleocapsid protein, *J. Virol.* 62 (1988) 4288–4295.
- [38] K.A. Spencer, M. Dee, P. Britton, J.A. Hiscox, Role of phosphorylation clusters in the biology of the coronavirus infectious bronchitis virus nucleocapsid protein, *Virol* 370 (2008) 373–381.
- [39] P. Masters, Localization of an RNA-binding domain in the nucleocapsid protein of the coronavirus mouse hepatitis virus, *Arch. Virol.* 125 (1992) 141–160, <https://doi.org/10.1007/BF01309634>.
- [40] H. Luo, Q. Chen, J. Chen, K. Chen, X. Shen, H. Jiang, The nucleocapsid protein of SARS coronavirus has a high binding affinity to the human cellular heterogeneous nuclear ribonucleoprotein A1, *FEBS Lett.* 579 (2005) 2623–2628, <https://doi.org/10.1016/j.febslet.2005.03.080>.
- [41] G.W. Nelson, S.A. Stohlman, S.M. Tahara, High affinity interaction between nucleocapsid protein and leader/intergenic sequence of mouse hepatitis virus RNA, *J. Gen. Virol.* 81 (2000) 181–188.
- [42] Chung-ke Chang, et al., Transient oligomerization of the SARS-CoV N protein—implication for virus ribonucleoprotein packaging, *PLoS One* 8 (5) (2013).
- [43] T.Y. Peng, K.R. Lee, W.Y. Tarn, Phosphorylation of the arginine/serine dipeptide-rich motif of the severe acute respiratory syndrome coronavirus nucleocapsid protein modulates its multimerization, translation inhibitory activity and cellular localization, *FEBS J.* 275 (2008) 4152–4163, <https://doi.org/10.1111/j.1742-4658.2008.06564.x>.
- [44] H. Luo, F. Ye, K. Chen, X. Shen, H. Jiang, SR-rich motif plays a pivotal role in recombinant SARS coronavirus nucleocapsid protein multimerization, *Biochemistry* 44 (2005) 15351–15358, <https://doi.org/10.1021/bi051122c>.
- [45] I.M. Yu, M.L. Oldham, J. Zhang, J. Chen, Crystal structure of the severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein dimerization domain reveals evolutionary linkage between corona- and arteriviridae, *J. Biol. Chem.* 281 (2006) 17134–17139, <https://doi.org/10.1074/jbc.M602107200>.
- [46] C.Y. Chen, C.K. Chang, Y.W. Chang, S.C. Sue, H.I. Bai, L. Riang, C.D. Hsiao, T. H. Huang, Structure of the SARS coronavirus nucleocapsid protein RNA-binding dimerization domain suggests a mechanism for helical packaging of viral RNA, *J. Mol. Biol.* 368 (2007) 1075–1086, <https://doi.org/10.1016/j.jmb.2007.02.069>.
- [47] Y.S. Lo, S.Y. Lin, S.M. Wang, C.T. Wang, Y.L. Chiu, T.H. Huang, M.H. Hou, Oligomerization of the carboxyl terminal domain of the human coronavirus 229E nucleocapsid protein, *FEBS Lett.* 587 (2013) 120–127, <https://doi.org/10.1016/j.febslet.2012.11.016>.
- [48] M. Takeda, C.K. Chang, T. Ikeya, P. Guntert, Y.H. Chang, Y.L. Hsu, T.H. Huang, M. Kainosho, Solution structure of the C-terminal dimerization domain of SARS coronavirus nucleocapsid protein solved by the SAIL-NMR method, *J. Mol. Biol.* 380 (2008) 608–622, <https://doi.org/10.1016/j.jmb.2007.11.093>.
- [49] Y. Huang, Z.Y. Yang, W.P. Kong, G.J. Nabel, Generation of synthetic severe acute respiratory syndrome coronavirus pseudoparticles: implications for assembly and vaccine production, *J. Virol.* 78 (2004) 12557–12565, <https://doi.org/10.1128/JVI.78.22.12557-12565.2004>.
- [50] Ying-Tzu Tseng, et al., Self-assembly of severe acute respiratory syndrome coronavirus membrane protein, *J. Biol. Chem.* 285 (17) (2010) 12862–12872.
- [51] C. Risco, I.M. Antón, L. Enjuanes, J.L. Carrascosa, The transmissible gastroenteritis coronavirus contains a spherical core shell consisting of M and N proteins, *J. Virol.* 70 (1996) 4773–4777.
- [52] Runtao He, et al., Characterization of protein-protein interactions between the nucleocapsid protein and membrane protein of the SARS coronavirus, *Virus Res.* 105 (2) (2004) 121–125.
- [53] Xiaonan Fang, et al., Peptide domain involved in the interaction between membrane protein and nucleocapsid protein of SARS-associated coronavirus, *BMB Rep.* 38 (4) (2005) 381–385.
- [54] Haibin Luo, et al., Severe acute respiratory syndrome coronavirus membrane protein interacts with nucleocapsid protein mostly through their carboxyl termini by electrostatic attraction, *Int. J. Biochem. Cell Biol.* 38 (4) (2006) 589–599.
- [55] R. He, F. Dobie, M. Ballantine, A. Leeson, Y. Li, N. Bastien, T. Cutts, A. Andonov, J. Cao, T.F. Booth, et al., Analysis of multimerization of the SARS coronavirus nucleocapsid protein, *Biochem. Biophys. Res. Commun.* 316 (2004) 476–483, <https://doi.org/10.1016/j.bbrc.2004.02.074>.
- [56] Aiping Wu, et al., Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China, *Cell Host Microbe* 27 (2020) 325–328.
- [57] D. Escors, J. Ortego, H. Laude, L. Enjuanes, The membrane M protein carboxy terminus binds to transmissible gastroenteritis coronavirus core and contributes to core stability, *J. Virol.* 75 (2001) 1312–1324, <https://doi.org/10.1128/JVI.75.3.1312-1324.2001>.
- [58] L. Kuo, P.S. Masters, Genetic evidence for a structural interaction between the carboxy termini of the membrane and nucleocapsid proteins of mouse hepatitis virus, *J. Virol.* 76 (2002) 4987–4999, <https://doi.org/10.1128/JVI.76.10.4987-4999.2002>.
- [59] M.A. Marra, S.J. Jones, C.R. Astell, R.A. Holt, A. Brooks-Wilson, Y.S. Butterfield, J. Khattra, J.K. Asano, S.A. Barber, S.Y. Chan, et al., The genome sequence of the SARS-associated coronavirus, *Science* 300 (2003) 1399–1404, <https://doi.org/10.1126/science.1085953>.
- [60] P.A. Rota, M.S. Oberste, S.S. Monroe, W.A. Nix, R. Campagnoli, J.P. Icenogle, S. Penaranda, B. Bankamp, K. Maher, M.H. Chen, S. Tong, et al., Characterization of a novel coronavirus associated with severe acute respiratory syndrome, *Science* 300 (2003) 1394–1399, <https://doi.org/10.1126/science.1085952>.
- [61] R.S. Baric, G.W. Nelson, J.O. Fleming, R.J. Deans, J.G. Keck, N. Casteel, S. A. Stohlman, Interactions between coronavirus nucleocapsid protein and viral RNAs: implications for viral transcription, *J. Virol.* 62 (1988) 4280–4287.
- [62] K. Narayanan, K.H. Kim, S. Makino, Characterization of N protein self-association in coronavirus ribonucleoprotein complexes, *Virus Res.* 98 (2003) 131–140, <https://doi.org/10.1016/j.virusres.2003.08.021>.
- [63] Silke Stertz, et al., The intracellular sites of early replication and budding of SARS-coronavirus, *Virology* 361 (2) (2007) 304–315.
- [64] Yingying Cong, et al., Nucleocapsid protein recruitment to replication-transcription complexes plays a crucial role in coronavirus life cycle, *J. Virol.* 94 (4) (2020).
- [65] Sarah C. Keane, David P. Giedroc, Solution structure of mouse hepatitis virus (MHV) nsp3a and determinants of the interaction with MHV nucleocapsid (N) protein, *J. Virol.* 87 (6) (2013) 3502–3515.
- [66] M.H. Verheije, M.C. Hagemeijer, M. Ulasli, F. Reggiori, P.J. Rottier, P.S. Masters, C.A. de Haan, The coronavirus nucleocapsid protein is dynamically associated with the replication-transcription complexes, *J. Virol.* 84 (2010) 11575–11579, <https://doi.org/10.1128/JVI.00569-10>.
- [67] Boyd Yount, et al., Reverse genetics with a full-length infectious cDNA of severe acute respiratory syndrome coronavirus, *Proc. Natl. Acad. Sci.* 100 (22) (2003) 12995–13000.
- [68] J.A. Hiscox, T. Wurm, L. Wilson, P. Britton, D. Cavanagh, G. Brooks, The coronavirus infectious bronchitis virus nucleoprotein localizes to the nucleolus, *J. Virol.* 75 (2001) 506–512.
- [69] R.R. Rowland, V. Chauhan, Y. Fang, A. Pekosz, M. Kerrigan, M.D. Burton, Intracellular localization of the severe acute respiratory syndrome coronavirus nucleocapsid protein: absence of nucleolar accumulation during infection and after expression as a recombinant protein in vero cells, *J. Virol.* 79 (2005) 11507–11512.
- [70] B. Zhou, J. Liu, Q. Wang, X. Liu, X. Li, P. Li, Q. Ma, C. Cao, The nucleocapsid protein of severe acute respiratory syndrome coronavirus inhibits cell cytokinesis and proliferation by interacting with translation elongation factor 1alpha, *J. Virol.* 82 (2008) 6962–6971, <https://doi.org/10.1128/JVI.00133-08>.
- [71] Torsten Wurm, et al., Localization to the nucleolus is a common feature of coronavirus nucleoproteins, and the protein may disrupt host cell division, *J. Virol.* 75 (19) (2001) 9345–9356.
- [72] Jae-Hwan You, Mark L. Reed, Julian A. Hiscox, Trafficking motifs in the SARS-coronavirus nucleocapsid protein, *Biochem. Biophys. Res. Commun.* 358 (4) (2007) 1015–1020.
- [73] M. Surjit, R. Kumar, R.N. Mishra, M.K. Reddy, V.T. Chow, S.K. Lal, The severe acute respiratory syndrome coronavirus nucleocapsid protein is phosphorylated and localizes in the cytoplasm by 14-3-3-mediated translocation, *J. Virol.* 79 (2005) 11476–11486, <https://doi.org/10.1128/JVI.79.17.11476-11486.2005>.
- [74] M. Surjit, B. Liu, S. Jameel, V.T. Chow, S.K. Lal, The SARS coronavirus nucleocapsid protein induces actin reorganization and apoptosis in COS-1 cells in the absence of growth factors, *Biochem. J.* 383 (2004) 13–18, <https://doi.org/10.1042/BJ20040984>.
- [75] X. Yan, Q. Hao, Y. Mu, K.A. Timani, L. Ye, Y. Zhu, J. Wu, Nucleocapsid protein of SARS-CoV activates the expression of cyclooxygenase-2 by binding directly to regulatory elements for nuclear factor-kappa B and CCAAT/enhancer binding protein, *Int. J. Biochem. Cell Biol.* 38 (2006) 1417–1428, <https://doi.org/10.1016/j.biocel.2006.02.003>.
- [76] S.A. Kopecky-Bromberg, L. Martinez-Sobrido, M. Frieman, R.A. Baric, P. Palese, Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists, *J. Virol.* 81 (2007) 548–557, <https://doi.org/10.1128/JVI.01782-06>.
- [77] X. Lu, J. Pan, J. Tao, D. Guo, SARS-CoV nucleocapsid protein antagonizes IFN-beta response by targeting initial step of IFN-beta induction pathway, and its C-terminal region is critical for the antagonism, *Virus Genes* 42 (2011) 37–45, <https://doi.org/10.1007/s11262-010-0544-x>.
- [78] N. Greenhalgh, R. Hull, E. Weston Hurst, The antiviral activity of acridines in eastern equine encephalomyelitis, rift valley fever and psittacosis in mice, and lymphogranuloma venereum in chick-embryos, *Br. J. Pharmacol. Chemother.* 11 (2) (1956) 220.
- [79] Hans Heymann, et al., The inhibition of ribonuclease by acidic polymers and their use as possible antiviral agents, *Arch. Biochem.* 73 (2) (1958) 366–383.
- [80] Tadakazu Tsuji, Researches on chemotherapeutic drugs against viruses. XVII.: synthesis and antiviral properties of urazoles and related compounds, *Pharmaceutical bulletin* 2 (4) (1954) 403–411.
- [81] Erik De Clercq, Antiviral drugs in current clinical use, *J. Clin. Virol.* 30 (2) (2004) 115–133.
- [82] Erik De Clercq, Guangdi Li, Approved antiviral drugs over the past 50 years, *Clin. Microbiol. Rev.* 29 (3) (2016) 695–747.
- [83] Jason D. Graci, Craig E. Cameron, Mechanisms of action of ribavirin against distinct viruses, *Rev. Med. Virol.* 16 (1) (2006) 37–48.
- [84] Roberts Angus Smith, William Kirkpatrick, Ribavirin: A Broad Spectrum Antiviral Agent, Academic Press, Inc, 111 Fifth Avenue, New York, NY 10003, USA, 1980.
- [85] Shane Crotty, Craig E. Cameron, Raul Andino, RNA virus error catastrophe: direct molecular test by using ribavirin, *Proc. Natl. Acad. Sci.* 98 (12) (2001) 6895–6900.
- [86] Julie K. Pfeiffer, Karla Kirkegaard, A single mutation in poliovirus RNA-dependent RNA polymerase confers resistance to mutagenic nucleotide analogs via increased fidelity, *Proc. Natl. Acad. Sci.* 100 (12) (2003) 7289–7294.
- [87] Mark Von Itzstein, et al., Rational design of potent sialidase-based inhibitors of influenza virus replication, *Nature* 363 (6428) (1993) 418–423.

- [88] Olivier Ferraris, Bruno Lina, Mutations of neuraminidase implicated in neuraminidase inhibitors resistance, *J. Clin. Virol.* 41 (1) (2008) 13–19.
- [89] Shuji Hatakeyama, et al., Emergence of influenza B viruses with reduced sensitivity to neuraminidase inhibitors, *Jama* 297 (13) (2007) 1435–1442.
- [90] Navin Horthongkham, et al., Epidemiological, clinical and virological characteristics of influenza B virus from patients at the hospital tertiary care units in Bangkok during 2011–2014, *PLoS One* 11 (7) (2016).
- [91] Mazhar Hussain, et al., Drug resistance in influenza A virus: the epidemiology and management, *Infection and drug resistance* 10 (2017) 121.
- [92] Mark R. Denison, et al., Coronaviruses: an RNA proofreading machine regulates replication fidelity and diversity, *RNA Biol.* 8 (2) (2011) 270–279.
- [93] Melanie A. Thompson, et al., Antiretroviral treatment of adult HIV infection: 2012 recommendations of the International Antiviral Society–USA panel, *Jama* 308 (4) (2012) 387–402.
- [94] Calvin J. Gordon, et al., Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency, *J. Biol. Chem.* 295 (2020) 6785–6797 (jbc-RA120).
- [95] Erik De Clercq, The design of drugs for HIV and HCV, *Nat. Rev. Drug Discov.* 6 (12) (2007) 1001–1018.
- [96] Bintou A. Ahidjo, et al., Current perspective of antiviral strategies against COVID-19, *ACS infectious diseases* 6 (7) (2020) 1624–1634, <https://doi.org/10.1021/acscinfed.0c00236>.
- [97] Changhyun Roh, A facile inhibitor screening of SARS coronavirus N protein using nanoparticle-based RNA oligonucleotide, *Int. J. Nanomedicine* 7 (2012) 2173–2179, <https://doi.org/10.2147/IJN.S31379>.
- [98] Chung-ke Chang, et al., Structure-based virtual screening and experimental validation of the discovery of inhibitors targeted towards the human coronavirus nucleocapsid protein, *Mol. BioSyst.* 12 (1) (2016) 59–66.
- [99] Qiulong Huang, et al., Structure of the N-terminal RNA-binding domain of the SARS CoV nucleocapsid protein, *Biochemistry* 43 (20) (2004) 6059–6063.
- [100] Shan-Meng Lin, et al., Structure-based stabilization of non-native protein–protein interactions of coronavirus nucleocapsid proteins in antiviral drug design, *J. Med. Chem.* 63 (6) (2020) 3131–3141.
- [101] T.R. Ruch, C.E. Machamer, The coronavirus E protein: assembly and beyond, *Viruses* 4 (2012) 363–382, <https://doi.org/10.3390/v4030363>.
- [102] Y.L. Siu, K.T. Teoh, J. Lo, C.M. Chan, F. Kien, N. Escriou, S.W. Tsao, J.M. Nicholls, R. Altmeyer, J.S. Peiris, et al., The M, E, and N structural proteins of the severe acute respiratory syndrome coronavirus are required for efficient assembly, trafficking, and release of virus-like particles, *J. Virol.* 82 (2008) 11318–11330, <https://doi.org/10.1128/JVI.01052-08>.