Role of 1'-Ribose Cyano Substitution for Remdesivir to Effectively Inhibit Nucleotide Addition and Proofreading in SARS-CoV-2 Viral RNA Replication

Lu Zhang[1,*], Peter Pak-Hang Cheung[2,*] and Xuhui Huang[2,*]

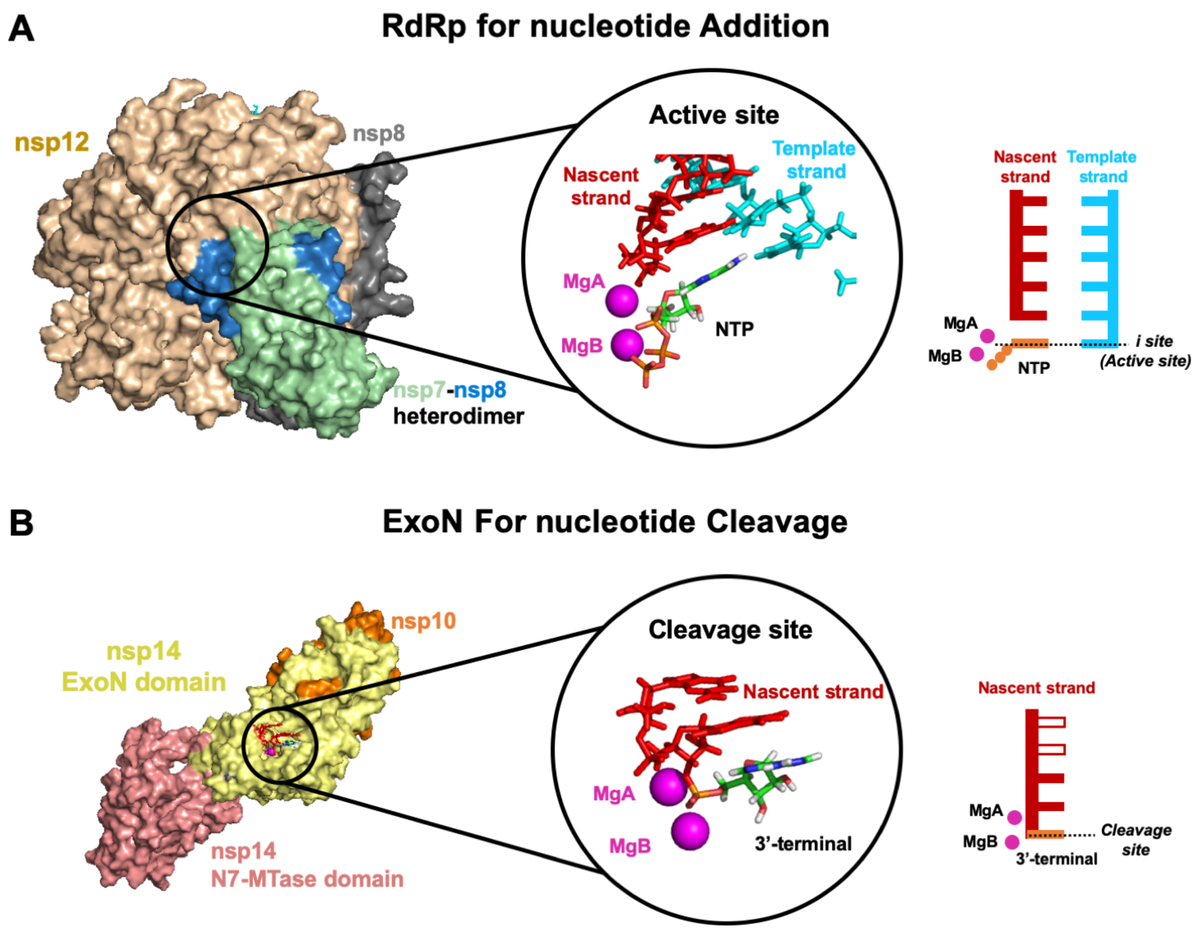
[1] State Key Laboratory of Structural Chemistry, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences, Fuzhou, Fujian, China

[2]Department of Chemistry, The Hong Kong University of Science and Technology

1. Introduction

The 2019 novel coronavirus (COVID-19 coronavirus (CoV) or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)) has spread rapidly to cause serious outbreaks and eventually a global pandemic. Antiviral agents are urgently needed to treat COVID-19 patients. Unfortunately, it could take years to develop new interventions. Hence, repurposing clinically approved or investigative drugs for other diseases provides a promising approach to develop COVID-19 treatments.

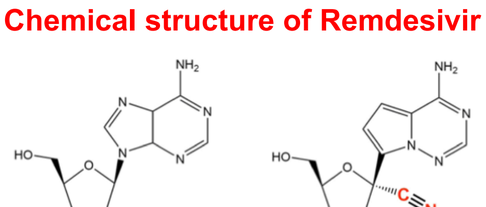
SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) is a promising but challenging drug target due to its intrinsic proofreading exoribonuclease (ExoN). Remdesivir targeting SARS-CoV-2 RdRp exerts high drug efficacy *in vitro* and *in vivo*. However, the molecular mechanisms underlying this delayed termination induced by Remdesivir remain largely elusive. Furthermore, it is unclear how Remdesivir can evade the proofreading activity of SARS-CoV-2 to prevent itself from being cleaved by ExoN.



2. Methods

To elucidate the mechanisms of Remdesivir inhibition on RdRp and ExoN, we conducted an aggregation of 24 μs all-atom molecular dynamics (MD) simulations of the nsp12-nsp7-nsp8 and nsp14-nsp10 complexes in SARS-CoV-2. Structural analysis were performed when Remdesivir is at different sites of nascent strand in RdRp, in comparison with the scenario of the wildtype-RNA.

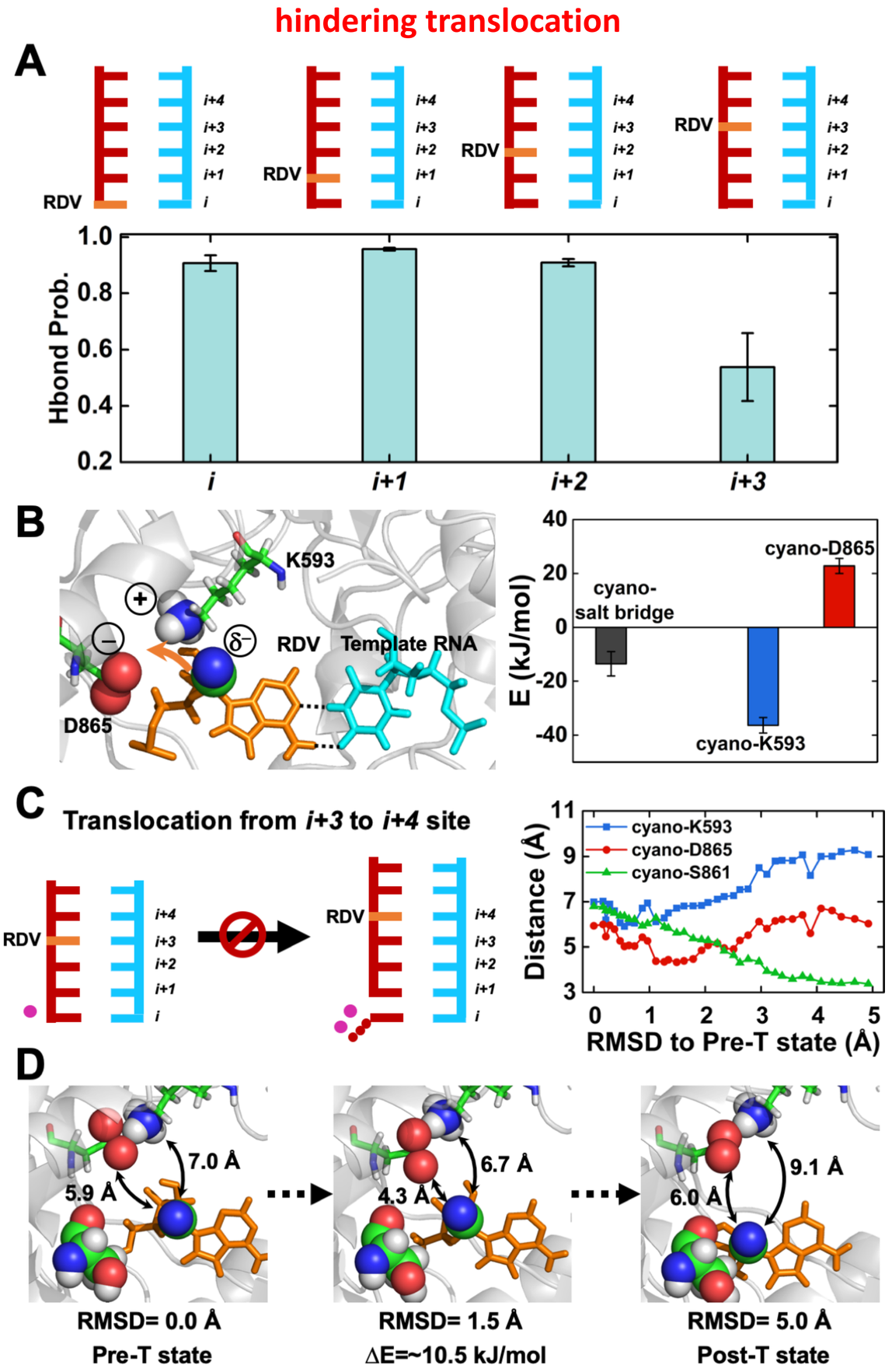
Chemical structure of Remdesivir



3. Results

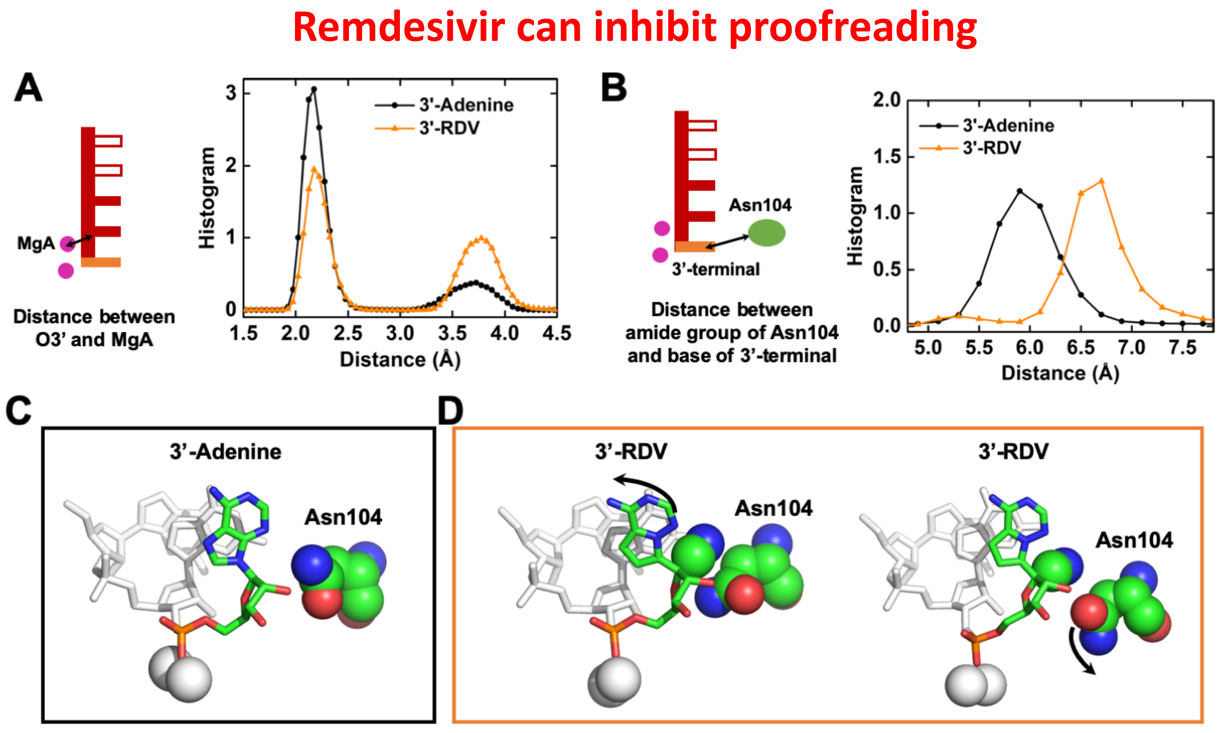
Remdesivir at the 3'-terminal of nascent RNA strand does not impact nucleotide addition

Remdesivir can abolish nucleotide addition in RdRp by hindering translocation



The translocation is hindered due to unfavorable interactions between the polar 1'-cyano group of Remdesivir at *i+3* site and a salt bridge formed by Asp865 and Lys593.

Remdesivir can inhibit proofreading



Remdesivir destabilizes the cleavage site due to the steric clash with the Asn104.

4. Conclusions

1. Remdesivir's 1'-cyano group of possesses the dual role of inhibiting nucleotide addition and proofreading.
2. When Remdesivir locates at an upstream site in RdRp, the 1'-cyano group causes instability via its electrostatic interactions with a salt bridge (Asp865-Lys593), which subsequently halts translocation. This leads to a delayed chain termination of RNA extension, which also subsequently reduces the likelihood for Remdesivir to be cleaved by ExoN acting on the 3'-terminal nucleotide.
3. Remdesivir's 1'-cyano group can also disrupt the cleavage site of ExoN via steric interactions, leading to a further reduced cleavage efficiency.

A preprint of this manuscript has been deposited to bioRxiv with the manuscript number: BIORXIV/2020/063859.
The authors declare no competing interests.