

Identifying structural features of nucleotide analogues to overcome SARS-CoV-2 exonuclease activity

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Supplementary Material

Supplementary Figures

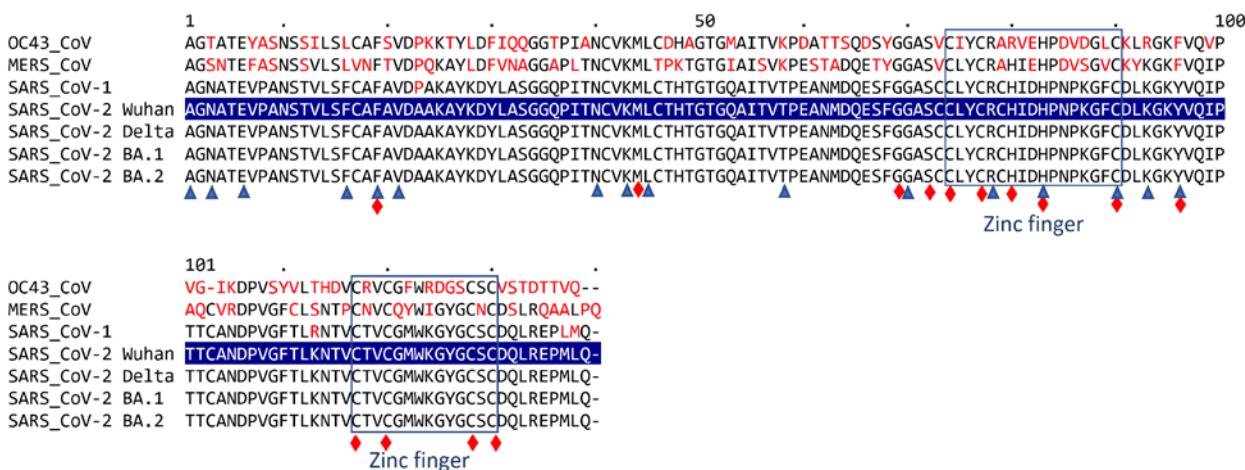


Figure S1. Protein sequence conservation in the Nsp10 in representative beta *Coronaviridae* strains. The Nsp10 of SARS-CoV-2 Wuhan-Hu-1 variant was used as a reference: amino acid differences compared to the reference sequence are highlighted in red. Zinc finger regions are boxed [1]. Residues interacting with Nsp14 are marked by blue triangles [2,3], the most important functional residues and those where mutations abolish the ExoN function or are lethal to SARS-CoV are shown as red diamonds [2-4]. The sequences were retrieved from GenBank: OC43-CoV (common cold; accession number YP_009924328), MERS-CoV (YP_009047238), SARS-CoV-1 (JX163928), and variants of SARS-CoV-2: Wuhan-Hu-1 (YP_009742617, original strain isolated in 2019), Delta (OM990852), and Omicron BA.1 (ON141240) and BA.2 (ON553707). Protein alignment was built using Clustal Omega [5,6], visualized by MView [6,7], and annotated.

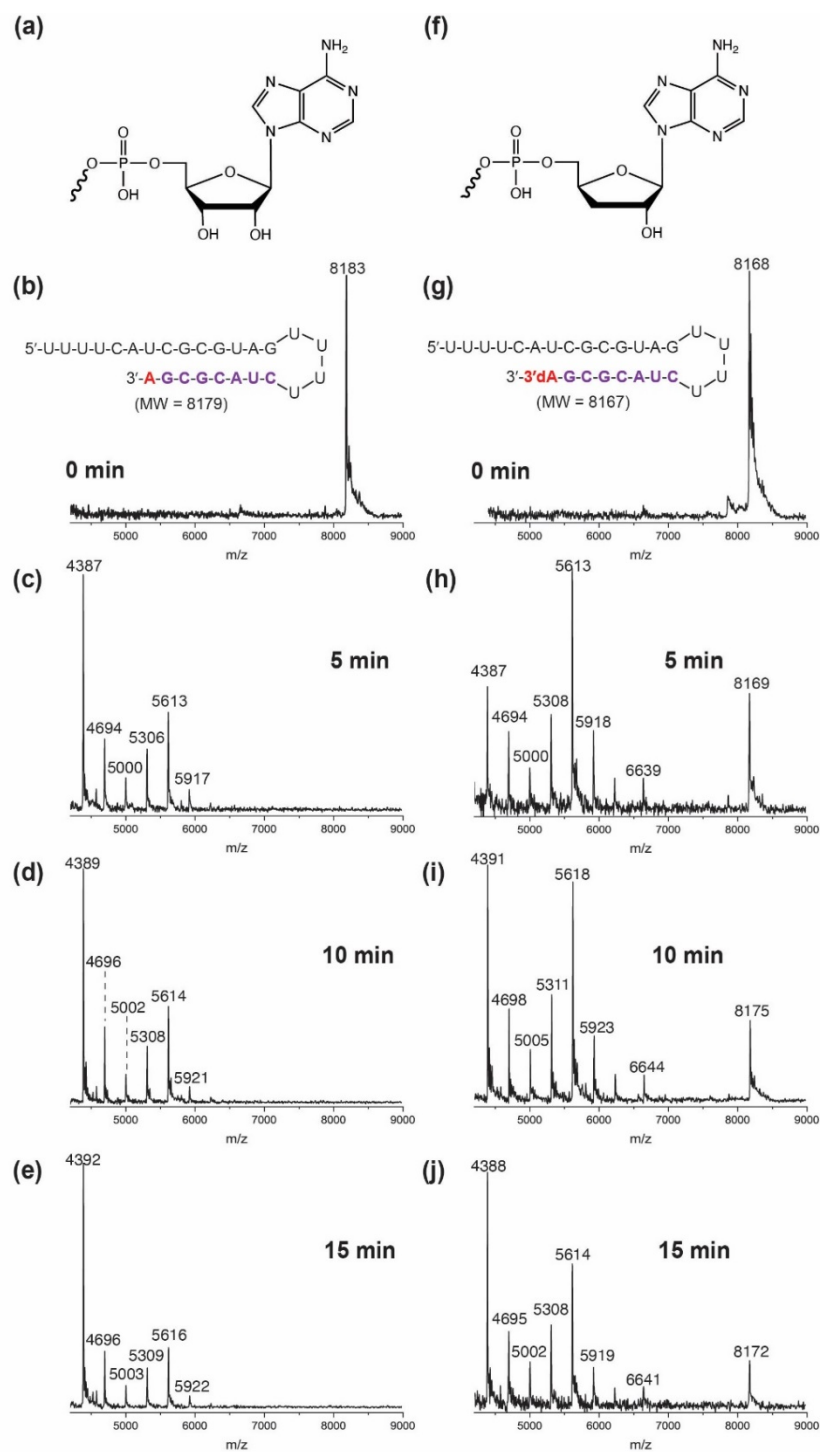


Figure S2. SARS-CoV-2 exonuclease activity with adenosine- or Cordycepin-terminated RNA for different incubation times. A mixture of 500 nM of adenosine-terminated template-loop-primer (sequences shown at the top of **b**) and SARS-CoV-2 pre-assembled exonuclease complex (Nsp14/Nsp10) was incubated at 37 °C for 5 min (**c**), 10 min (**d**), or 15 min (**e**). A mixture of 500 nM of Cordycepin-terminated template-loop-primer (sequences shown at the top of **g**) and SARS-CoV-2 pre-assembled exonuclease complex (Nsp14/Nsp10) was incubated at 37 °C for 5 min (**h**), 10 min (**i**), or 15 min (**j**). The intact RNAs (**b**, **g**) and their respective exonuclease reaction products (**c-e**, **h-j**) were analyzed by MALDI-TOF MS. The signal intensity was normalized to the highest peak.

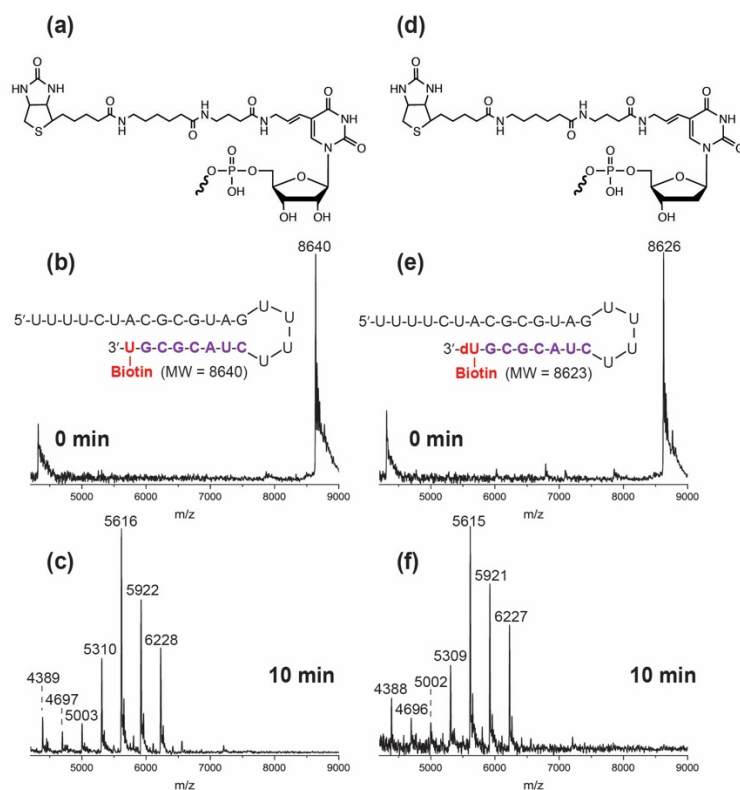


Figure S3. SARS-CoV-2 exonuclease activity with uridine analogue-terminated RNA. A mixture of 500 nM template-loop-primer terminated at its 3' end with either Biotin-16-U (**a**) or Biotin-16-dU (**d**) (sequences shown in **b**, **e**) and SARS-CoV-2 pre-assembled exonuclease complex (Nsp14/Nsp10) was incubated at 37 °C for 10 minutes. These intact RNAs (**b**, **e**) and their respective exonuclease reaction products (**c**, **f**) were analyzed by MALDI-TOF MS. The signal intensity was normalized to the highest peak.

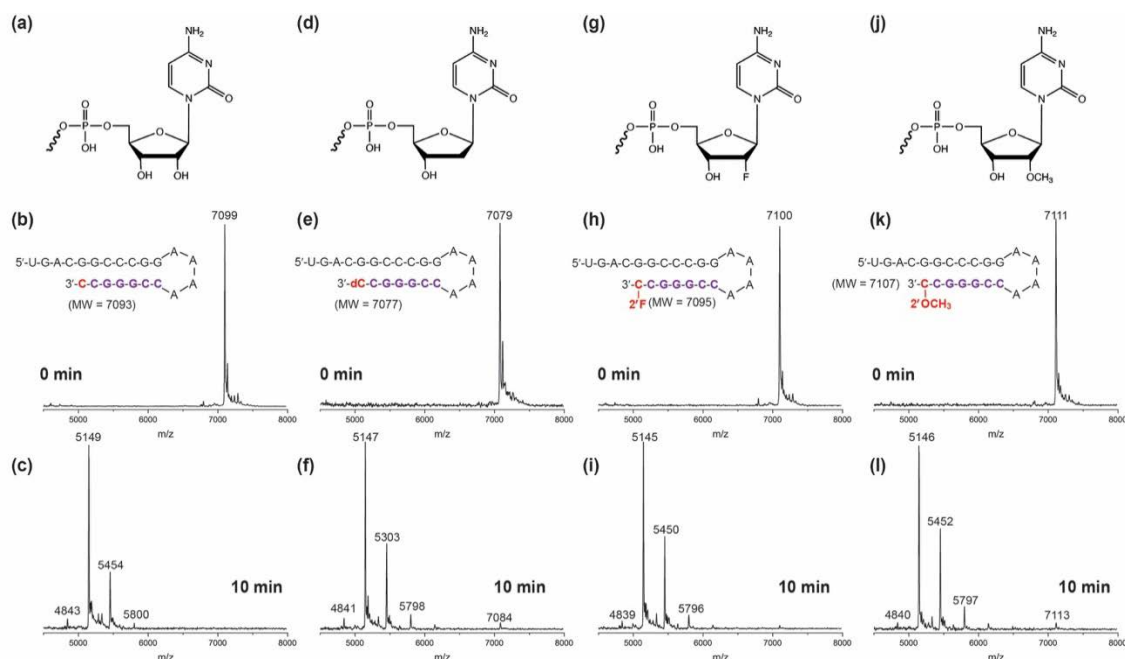


Figure S4. SARS-CoV-2 exonuclease activity with cytosine- or cytosine analogue-terminated RNA. A mixture of 500 nM of template-loop-primer terminated at its 3' end with either C (a), 2'-dC (d), 2'-F-2'-dC (g) or 2'-OMe-C (j) (sequences shown in b, e, h, k) and SARS-CoV-2 pre-assembled exonuclease complex (Nsp14/Nsp10) was incubated at 37 °C for 10 minutes. These intact RNAs (b, e, h, k) and their respective exonuclease reaction products (c, f, i, l) were analyzed by MALDI-TOF MS. The signal intensity was normalized to the highest peak.

Supplementary References

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