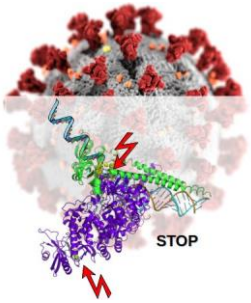
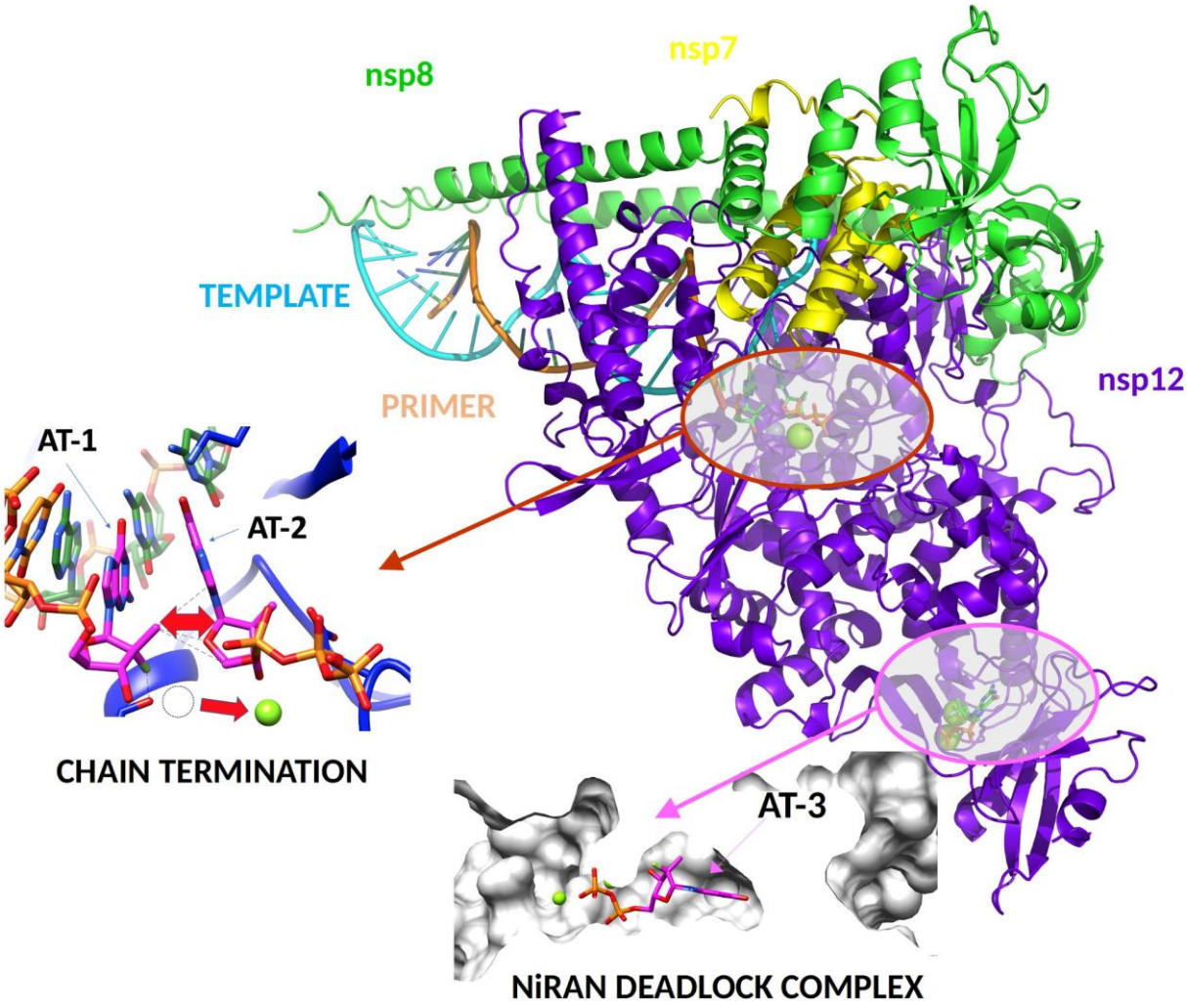


AT-527 stops SARS-CoV-2 replication by simultaneously targeting two active sites

There are few treatments and drug candidates directly targeting severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). Among those targeting the RNA polymerase complex, Favipiravir, Remdesivir and Molnupiravir have drawbacks, such as toxicity, a mutagenic potential to be monitored in the long term, or the still unknown possibility of generation of variants. Other drug candidates are inactivated by their excision of RNA promoted by the viral exonuclease nsp14 / nsp10.

The nucleotide analogue AT-527 is a promising candidate currently in phase II / III clinical trials for the treatment of COVID-19. AT-527 is a prodrug that is converted in cells to the nucleoside triphosphate, AT-9010, which lures viral RNA-dependent RNA polymerase (RdRp, nsp12), for incorporation into viral RNA without mutagenic effect.

The work presents a three-dimensional structure obtained by Cryo-EM at 2.98 Å resolution of the SARS-CoV-2 nsp12-nsp7- (nsp8) 2-RNA complex, showing AT-9010 bound to three sites of nsp12 , the core protein of the replicative complex.



In the RdRp active site of nsp12, an AT-9010 is incorporated into the 3 'end of the RNA product strand (**AT-1, figure**). Its modified ribose (2'-fluoro, 2'-methyl) prevents the correct alignment of the incoming NTP, in this case a second AT-9010 (**AT-2, figure**), causing the immediate stop of RNA synthesis . The third AT-9010 is linked to the N-terminal domain of nsp12 (**AT-3, figure**), known as the *Nidovirus RNA-polymerase Associated Nucleotidylation* (NiRAN) domain.

In the latter, and unlike natural NTPs, AT-9010 is in an orientation reversed to that observed in the active site of *SelO*-type enzymes, a pseudo-kinase showing partial structural homology to NiRAN. Its nucleobase unexpectedly occupies a previously unnoticed cavity. AT-9010 outperforms all natural nucleotides for NiRAN binding, inhibiting its essential nucleotidyltransferase activity for viral growth.

In addition, the chemical nature of the AT-9010 terminator slows down about 5 times the rate of its excision by nsp14 / nsp10.

The absence of toxicity and mutagenic potential of AT-527, its oral bioavailability, and its double mechanism of action on the active sites RdRp and NiRAN - making it more difficult to acquire viral resistance - therefore give this drug a promising potential against COVID-19, but also against all existing or future coronaviruses.

Reference: Shannon et al. Nature Communications, 2022, in press.

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