T-705-modified ssRNA In Complex With Lassa Virus Nucleoprotein Exhibits Nucleotide Splaying And Increased Water Influx Into The RNA-binding Pocket

Olaposi Omotuyi, Oyekanmi Nash, David Safronetz & Ayodeji Ojo

Olaposi Omotuyi: Center for Biocomputing and Drug Development, Adekunle Ajasin University, Akungba-Akoko, Nigeria,

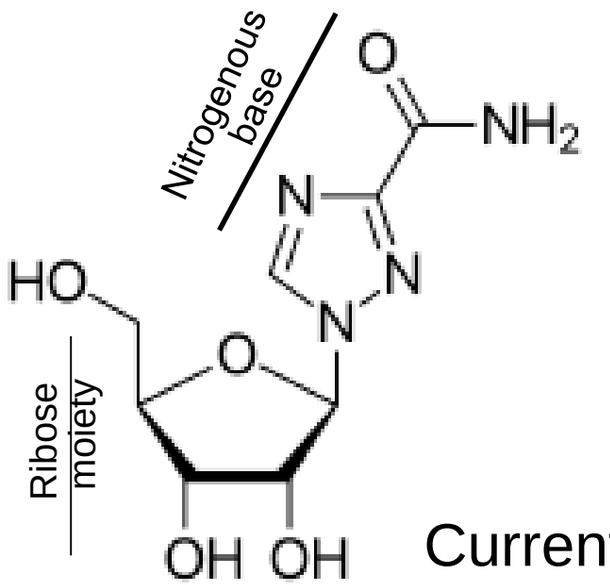
Oyekanmi Nash: Center for Genomics Research and Innovation, National Biotechnology Development Agency, Abuja, Nigeria

David Safronetz: Special Pathogens Program, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada

Ayodeji Ojo: Department of Public and Community Health, Liberty University, Lynchburg, Virginia, United States.

Background: The two woes of Ribavirin

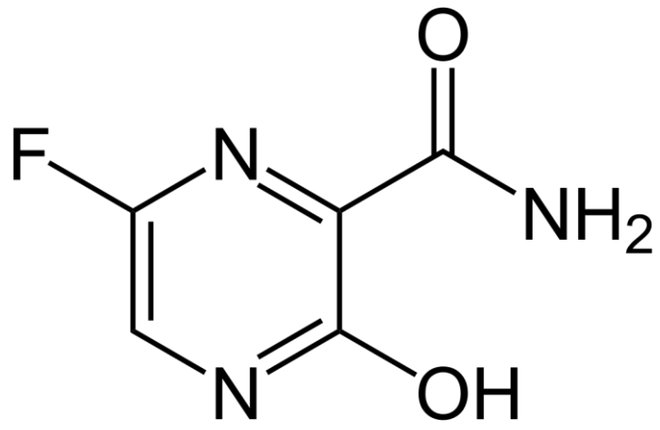
Whilst LASSARAB is underway as a promising biologics for LASV infection prevention (Abreu-Mota *et al*., 2018), former and current infections have relied exclusively on Ribavirin. The mechanism is thought to be interferon-gene upregulation, inhibition of host IMP dehydrogenase, and viral RNA-dependent RNA polymerase (Carrillo-Bustamante et al., 2017).



Ribavirin

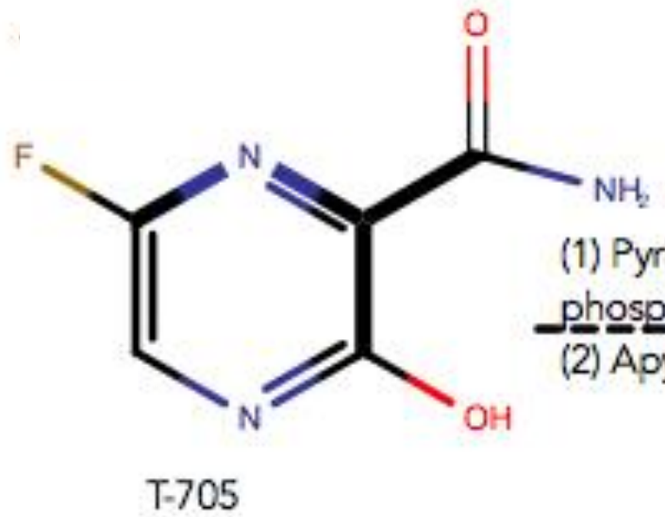
Many patients have benefitted from this treatment, especially when diagnosis and treatment is early. In Nigeria, and other West African countries plagued with LASV, diagnosis is often times very late and treatment even later; therefore constituting a major drawback to its use in these countries. Ribavirin is also associated with clinical toxicity (Gowen *et al.,* 2008).

Current research is geared towards investigating novel treatment options; ones with efficacy at every stage of infection and with reduced toxicity. One of the candidates under under investigation is Favipiravir (also called T-705). T-705 has demonstrable efficacy in chronically infected *cynomolgus macaques* (Rosenke *et al.,* 2018) and humans (Raabe *et al.,* 2017).



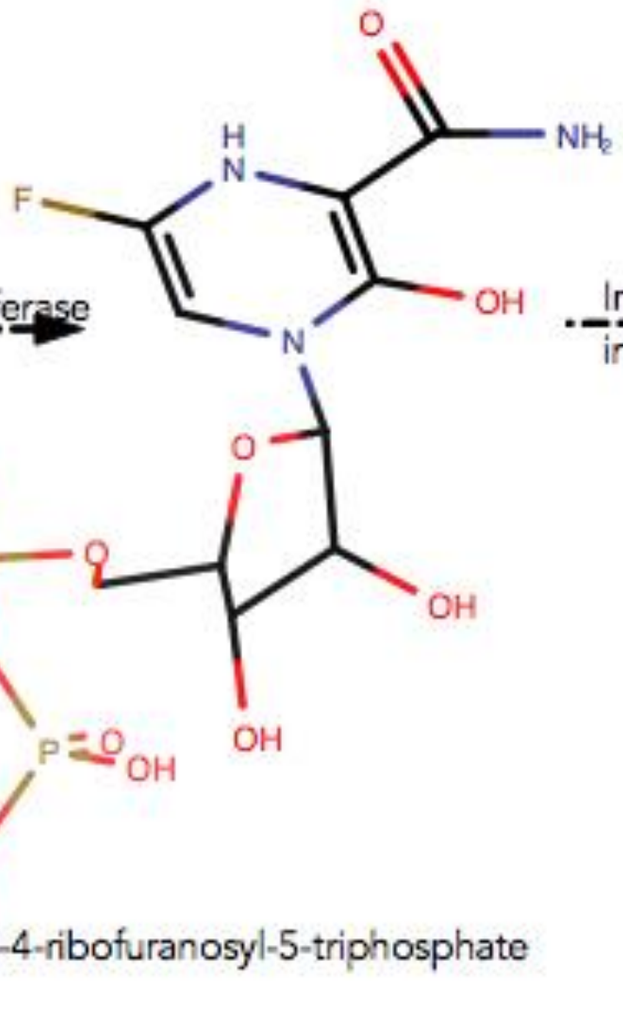
Favipiravir

T-705 Antiviral Mechanism



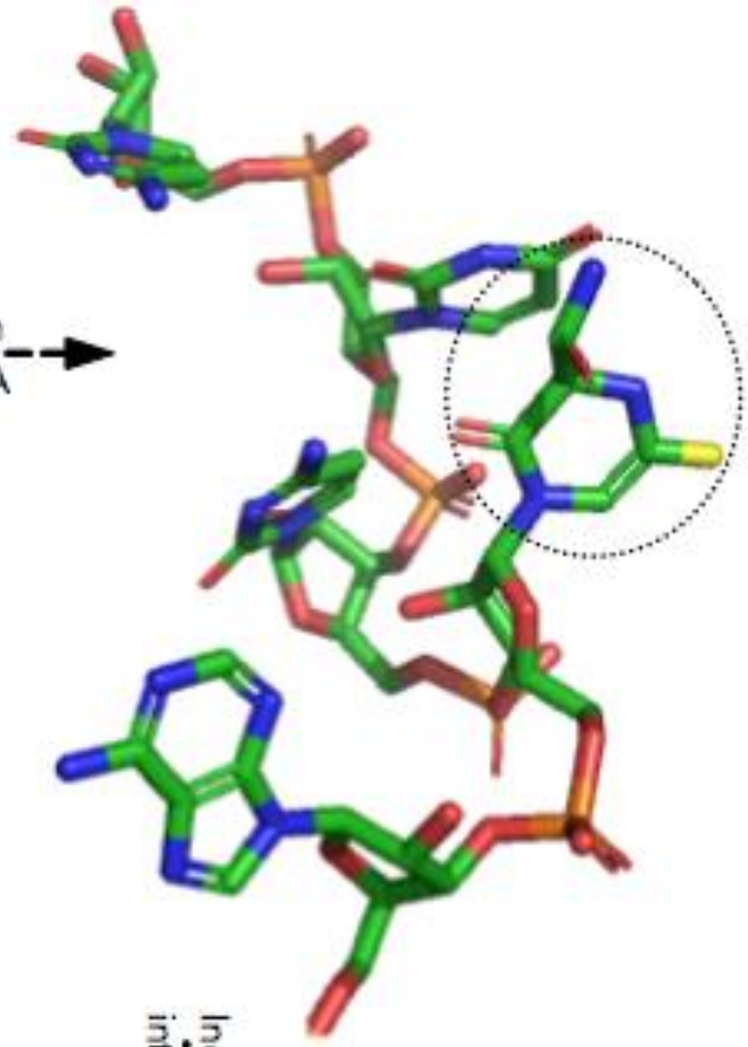
T-705

(1) Pyrimidine phosphoribosyltransferase
(2) Apyrase



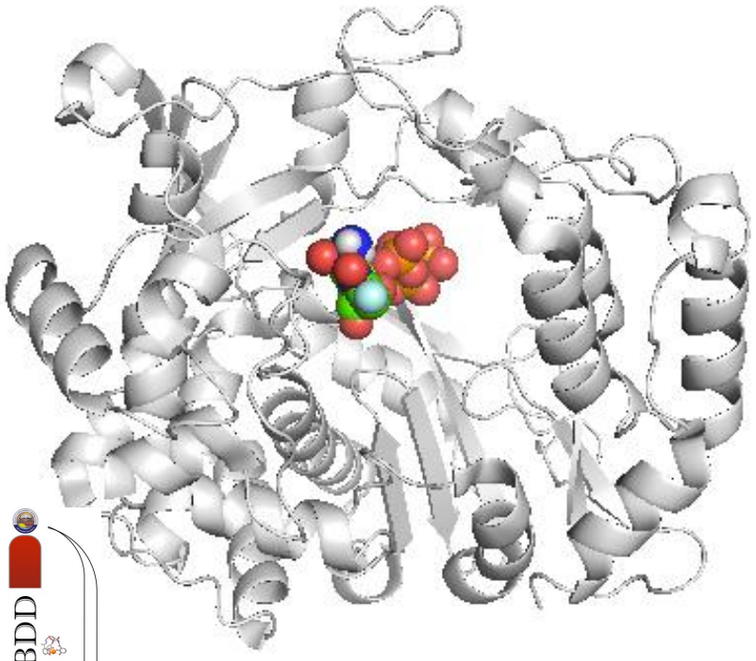
T-705-4-ribofuranosyl-5-triphosphate

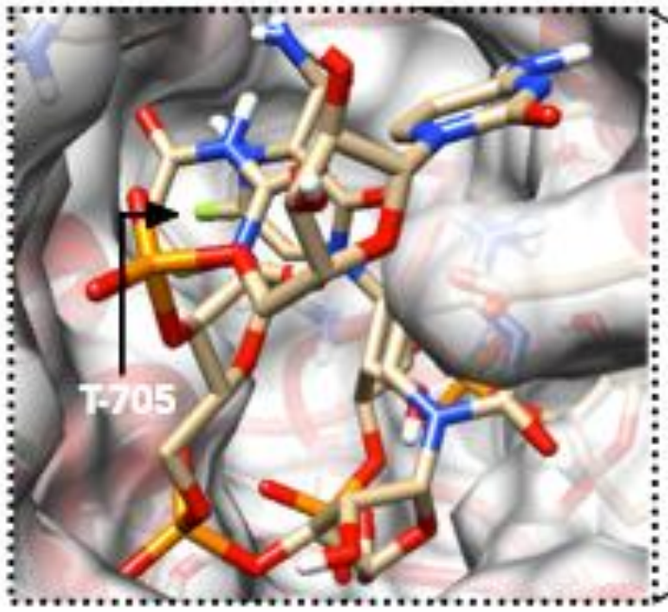
Incorporation into viral RNA

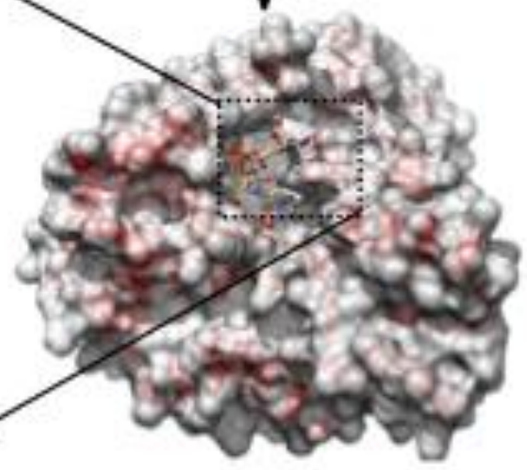


Incorporation into viral RNA

Inhibition of Catalytic domain of RdRp







Lassa nucleoprotein binds ssRNA

CBDD

Thinking Science Research Innovation

T-705-RTP is incorporated into LASV-ssRNA and now what?

The most important question in this study is “how does the presence of T-705 affect the structural and functional integrity of Lassa virus Nucleoprotein-ssRNA complex which ultimately accounts for its antiviral potency?”.

In terms of the function: LASV genome is bisegmented ambisense single-stranded RNA (ssRNA) which lacks the eukaryotic-type 7-methylguanylate (m7G) cap required for protection against host ribonuclease. During replication therefore, nascently formed single-stranded genomic (or the antigenomic) RNA is shielded from host ribonuclease by forming complex with the N-terminal domain of LASV nucleoprotein (NP) (Brunotte *et al.,* 2011).

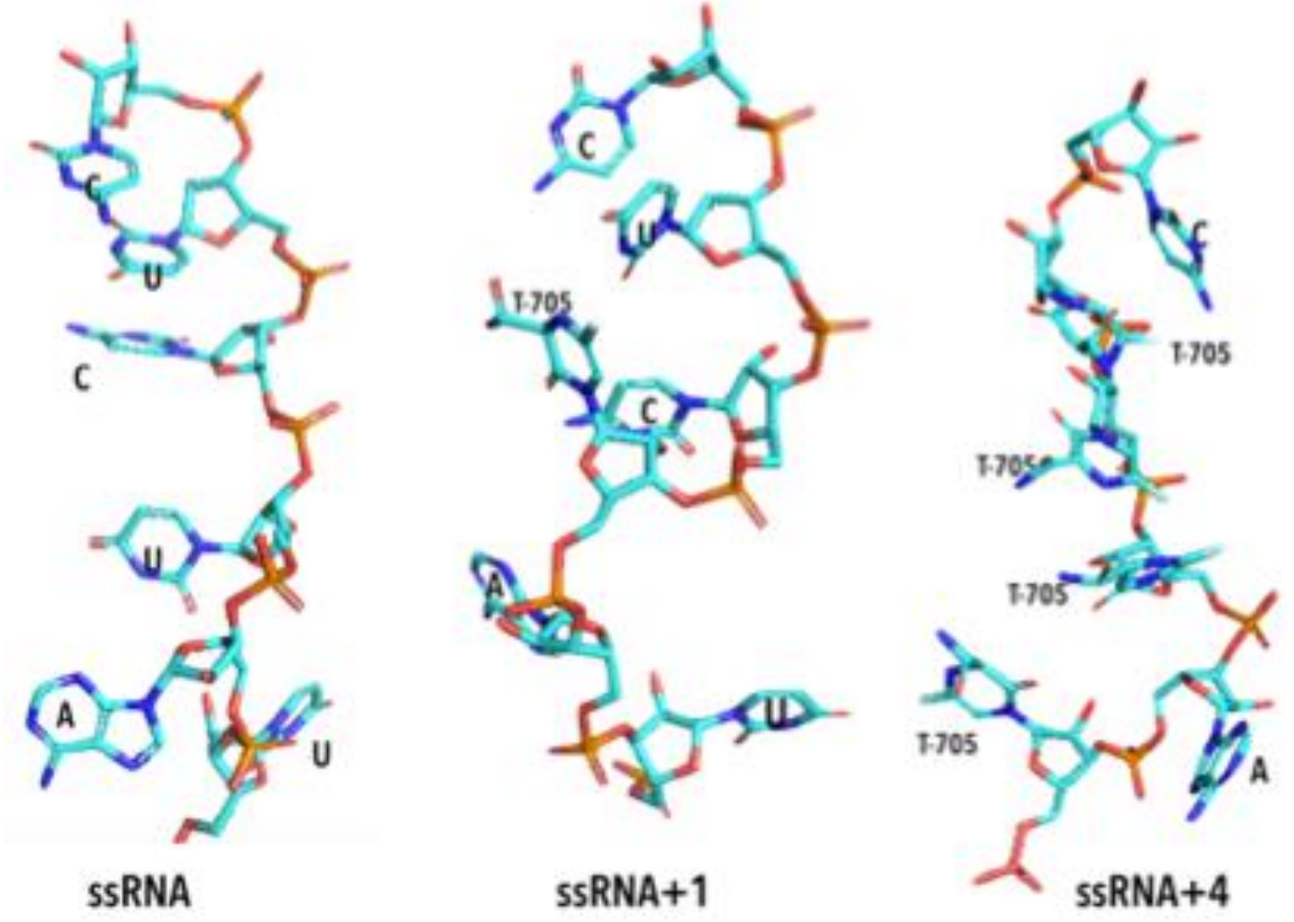
The research questions were; how does T-705 substitution in viral ssRNA affect

i. Torsion angles around ssRNA bases

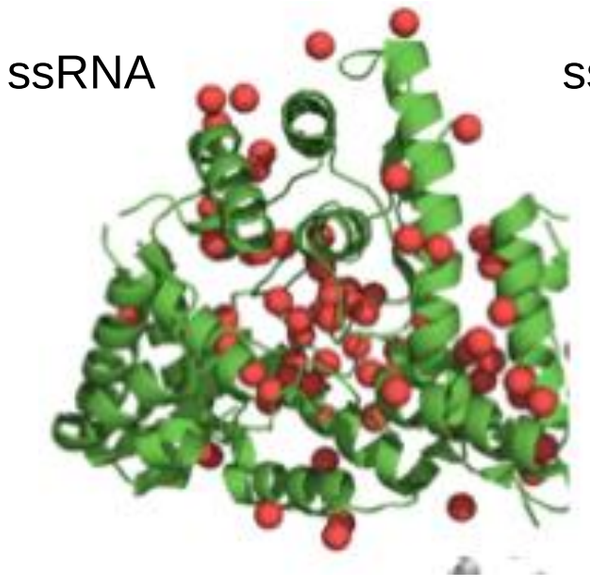
ii. Native interaction with Lassa virus Nucleoprotein

iii. The solvation of the ssRNA-binding pocket.

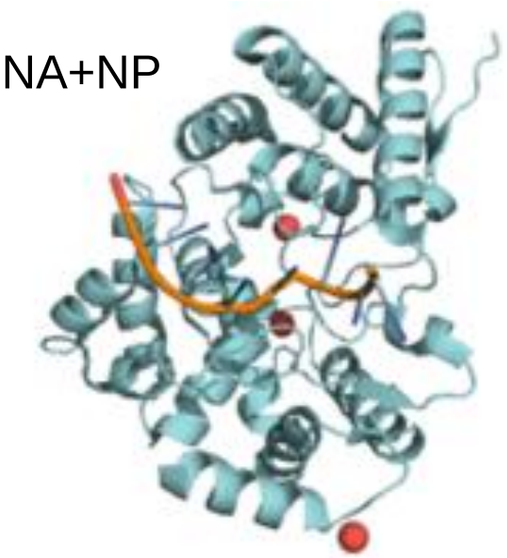
iv. The strength of interaction between ssRNA and nucleoprotein.



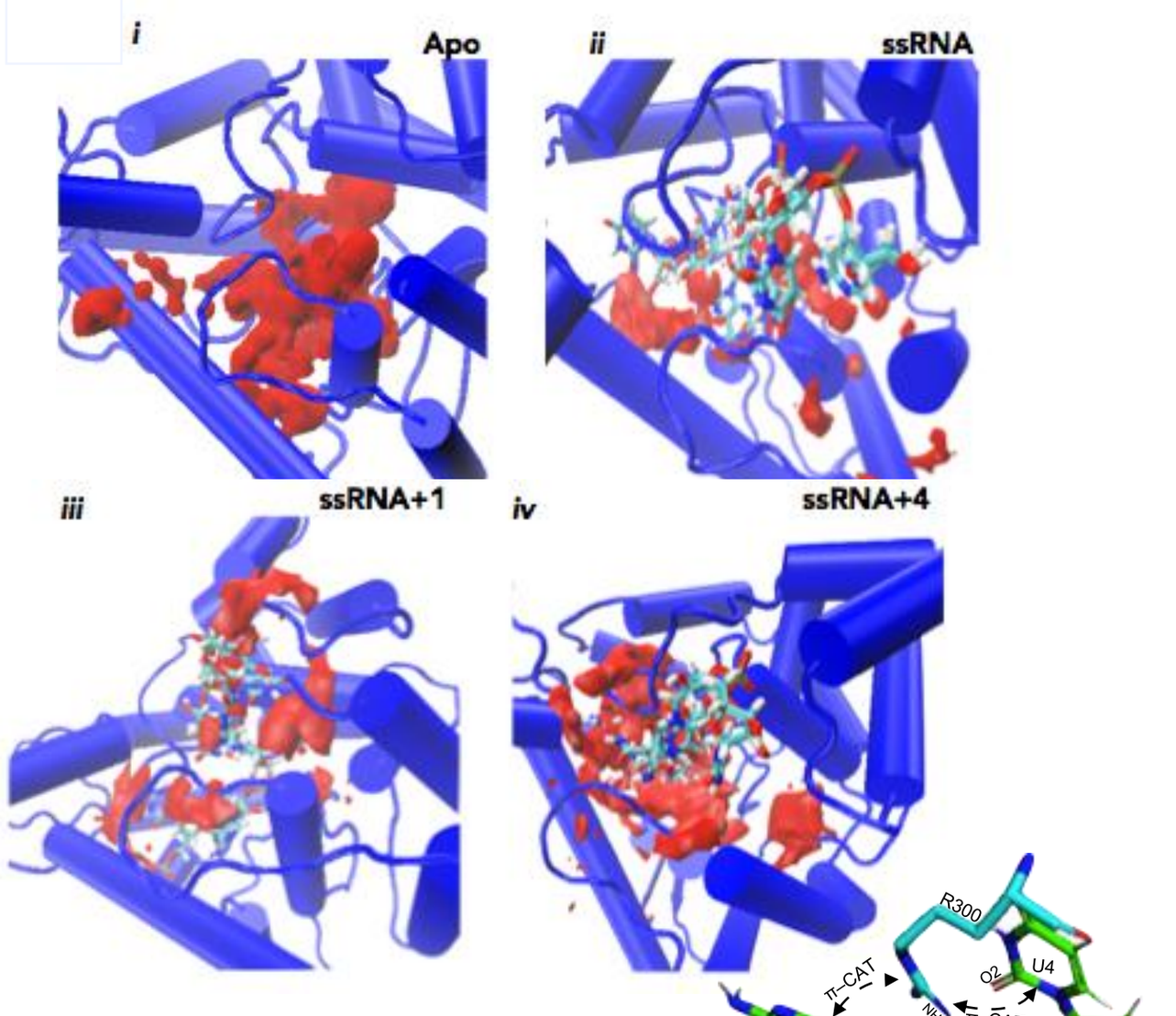
T-705-ssRNA promotes water permeation into the LASV-NP ssRNA binding cleft, alters torsion around the sugar-base bond and causes nucleotide splaying out

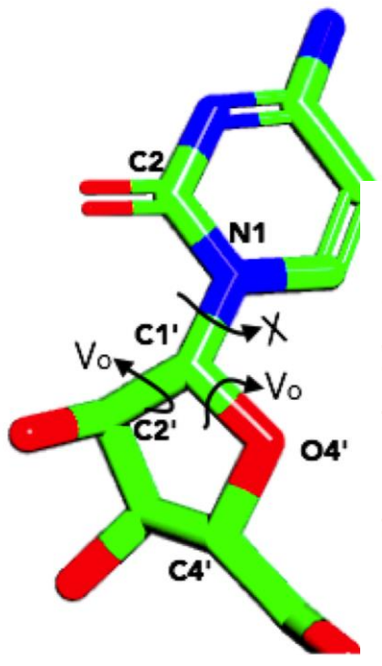


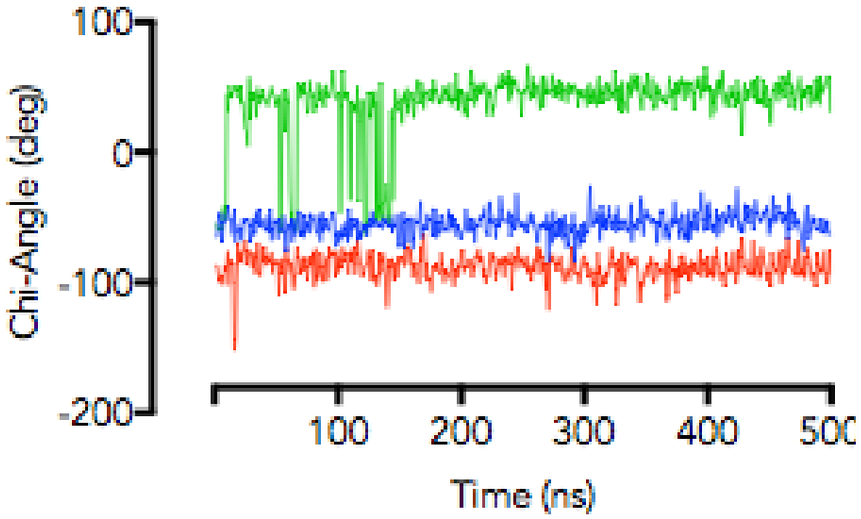
Highly solvated



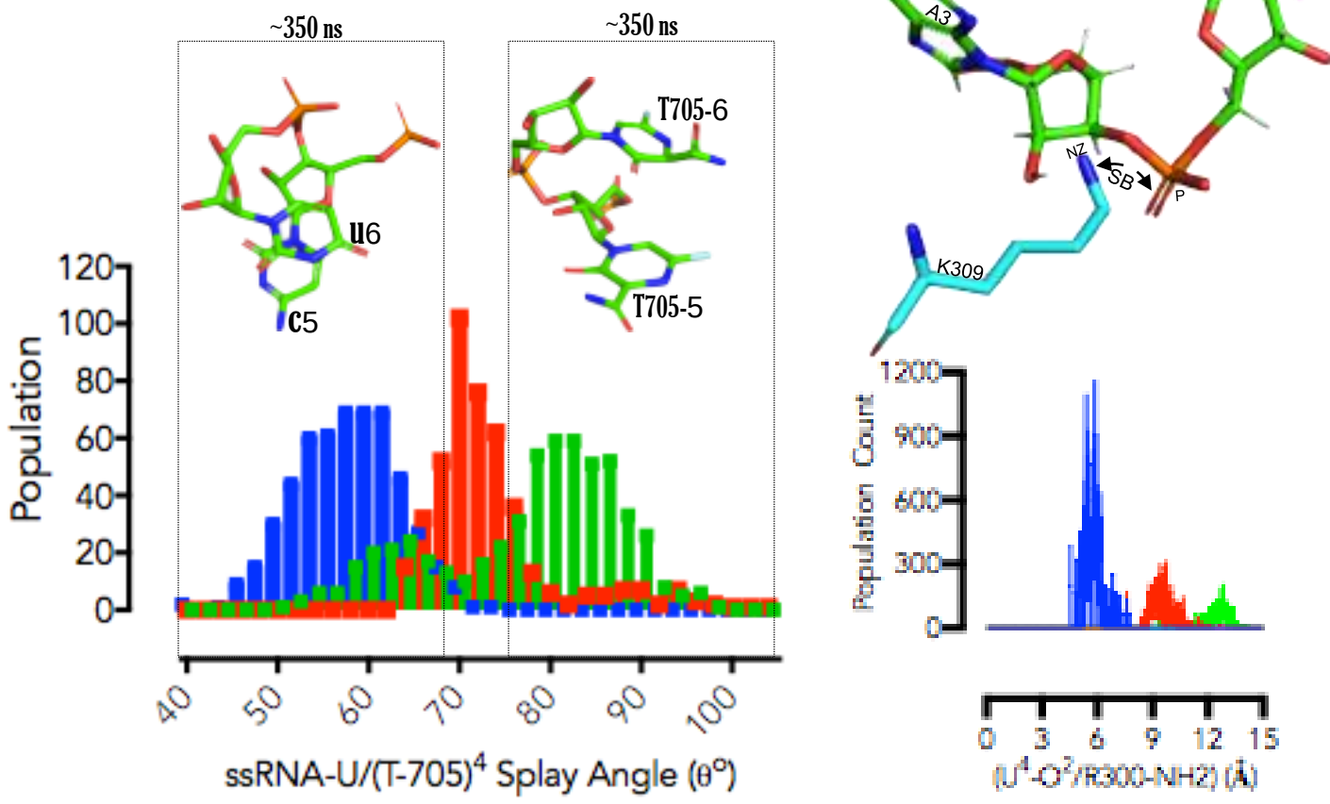
desolvated





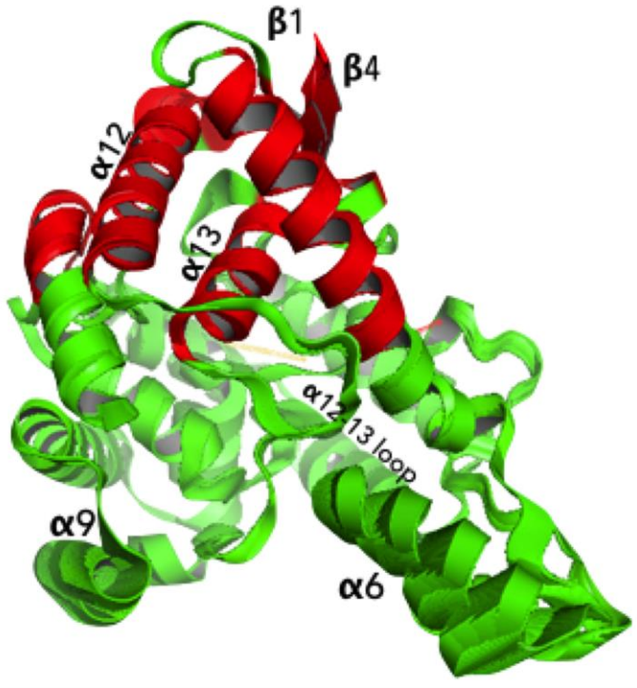


ssRNA+4
ssRNA+1
ssRNA+0



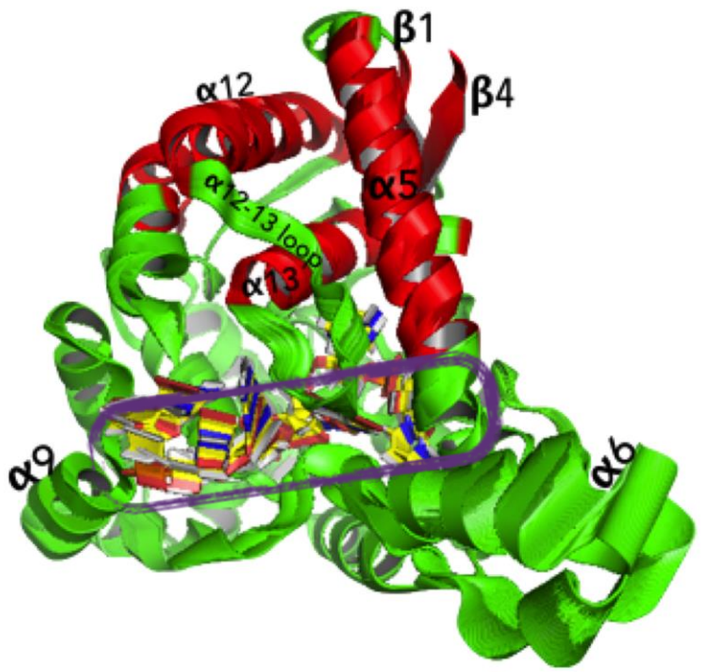
T-705-substitution in ssRNA destabilises LASV-NP binding and may result in ssRNA dissociation, thus exposing viral ssRNA to host RNases.

i



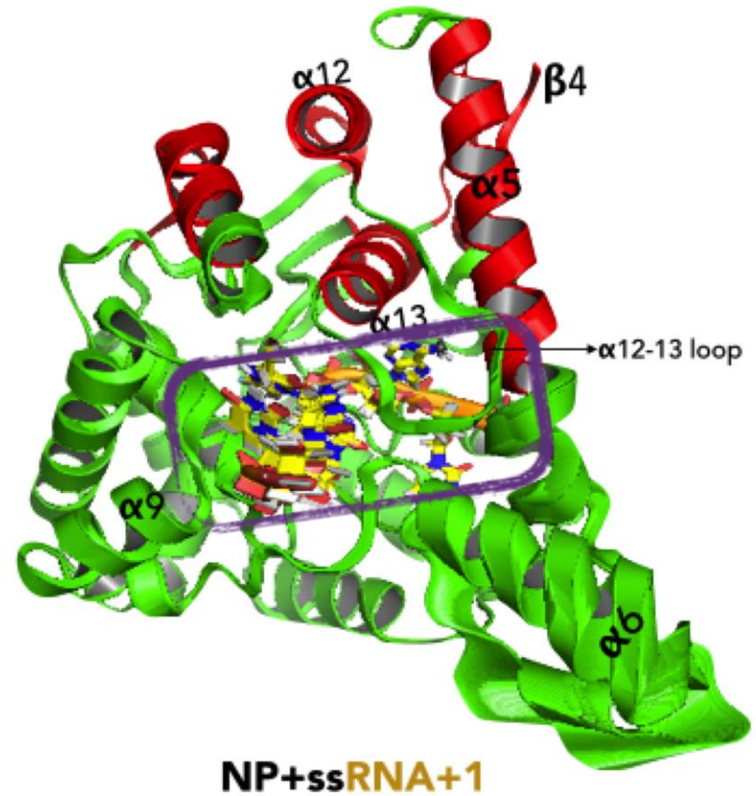
APO-NP

ii



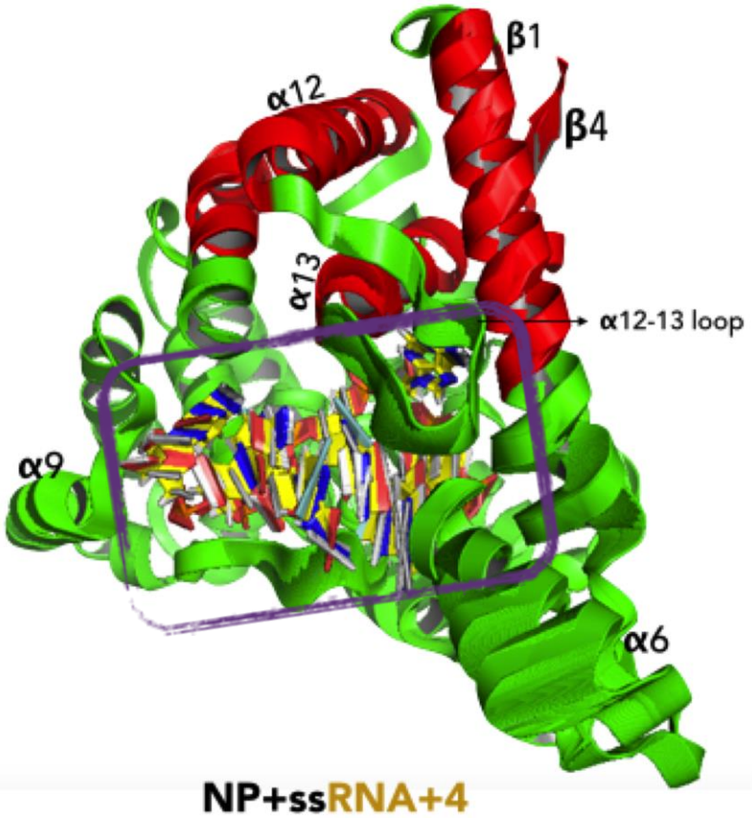
NP+ssRNA

iii



NP+ssRNA+1

iv



NP+ssRNA+4

Conclusions

- Evidence here strongly support the anti-Lassa virus potency of T-705
- Alteration of ssRNA structure and dynamical interaction with Lassa virus Nucleoprotein is a major contributor to its anti-Lassa virus activity.
- Specifically, multi-substituted ssRNA is associated with out-of-plane splaying of the nucleotides causing loss of base-stacking interaction.
- Lack of base-stacking interaction within the modified bases also result in loss of interaction with protein residues within Lassa virus nucleoprotein RNA pocket.
- With the loss of ssRNA-NP interaction within the pocket, influx of water is increased.
- Ultimately, the essential dynamics results show that T-705-modified ssRNA exhibits high entropy with a chance of complete dissociation under longer simulation time.

DOI: 10.1111/cbdd.13451

RESEARCH ARTICLE

WILEY

T-705-modified ssRNA in complex with Lassa virus nucleoprotein exhibits nucleotide splaying and increased water influx into the RNA-binding pocket

Olaposi I. Omotuyi[1] | Oyekanmi Nash[2] | David Safronetz[3] | Ayodeji A. Ojo[4] | Tomisin H. Ogunwa[1,5] | Niyi S. Adelakun[1]

Acknowledgments

Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria for BioMedical Research Grant (Grant Number: ADV/50/2016/000004).

First Bank Nigeria for providing Solar Power for analysis of simulation trajectories.

Dr. Hamada Tsuyoshi (Nagasaki University) for providing three GPUs and two HPCs used in this study.

Prof. Ueda Hiroshi (Nagasaki University Therapeutic Innovation Center, Nagasaki University) for providing one of the HPC hardware.