

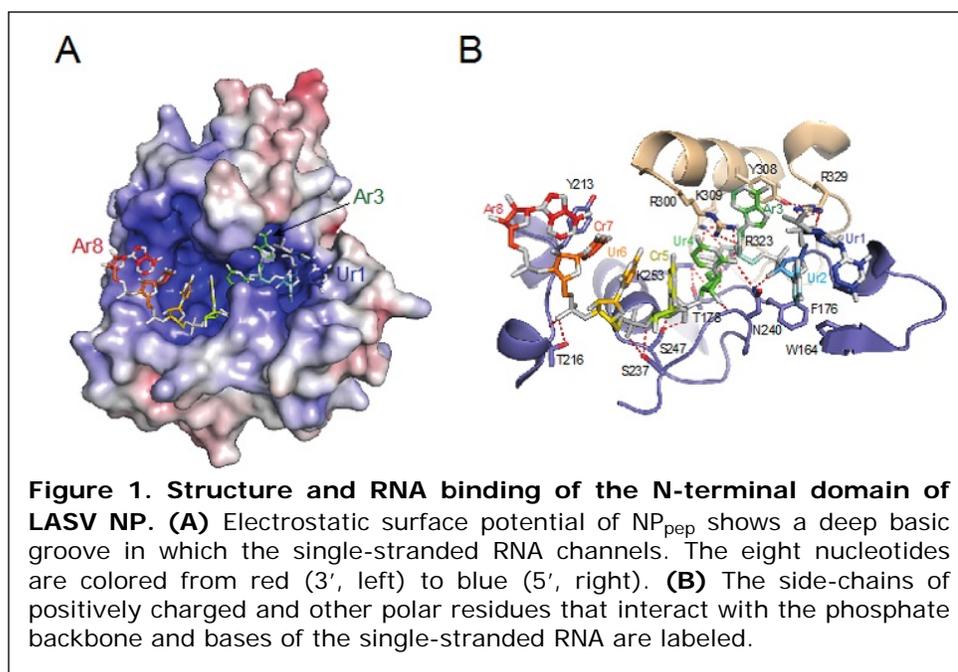
## The Lassa Virus Nucleoprotein Exhibits Conformational Control of Genome Binding

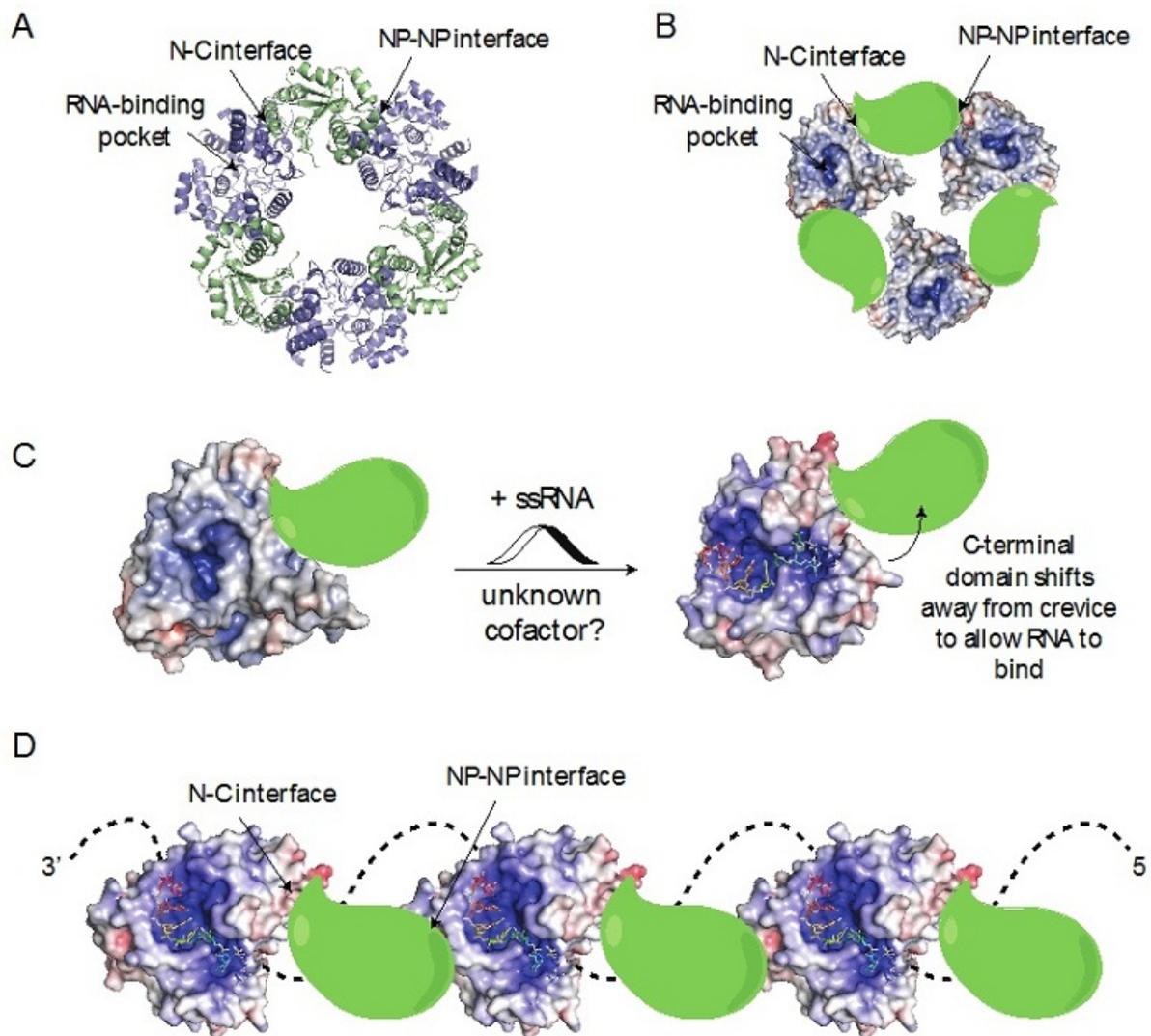
Lassa virus is endemic in Western Africa, and is the most common cause of viral hemorrhagic fever, infecting an estimated 300,000-500,000 people annually. It is also the hemorrhagic fever most frequently transported out of Africa to the United States and Europe. Understanding the key proteins of Lassa virus, and any Achilles heels written into their protein structures, will enable development of therapeutics for medical defense.

The genomic (and antigenomic) RNA of viruses like Ebola and measles, which encode some or all of their genes in the negative direction, do not exist as naked RNA. Instead, their RNAs are encapsidated by a viral nucleoprotein. During replication of these viruses, the nascent nucleoprotein (usually termed N) is bound by a cofactor (often a phosphoprotein termed P), which prevents polymerization of N and nonspecific encapsidation of host cellular RNAs in place of the desired viral RNAs. The resulting complex is termed N<sup>0</sup>-P, in which N<sup>0</sup> denotes RNA-free N. However, other negative-strand RNA viruses such as Lassa have surprisingly simple genomes. Lassa virus only encodes four proteins, and none of them is a "P". Hence, the mechanism by which this common hemorrhagic fever virus controls genome binding during infection is not yet understood. Our recent crystal structure of the RNA binding domain of the Lassa, termed NP, reveals that RNA binding may be controlled by conformational gating, rather than by association with a phosphoprotein.

Lassa NP has distinct N- and C-terminal domains connected by a flexible linker. Recent work demonstrated that unexpectedly, the C-terminal domain functions as an exonuclease<sup>1,2</sup> specific for double-stranded RNA (dsRNA)<sup>3</sup>. dsRNA is a key signature of virus infection and a powerful signal to the host's innate immune system to mount an antiviral response. Rapid digestion of dsRNA may allow the deadly virus and/or facilitate some other function in replication of the negative-sense genome. The structure of the full-length Lassa NP, determined in the absence of RNA, suggested the N-terminal domain contained an mRNA capping site and predicted the viral RNA would bind in the cleft between the two domains<sup>2</sup>.

Our structures of the N-terminal domain in complex with ssRNA indicate that, instead, this domain binds viral RNA and not mRNA cap. However, the full-length NP was crystallized as a trimer, and the interactions of the trimer appear to hold closed the RNA-binding cleft. We hypothesized that the trimeric form of NP may exist to prevent non-specific binding of cellular RNA (Figure 1).





**Figure 2. A model for arenavirus RNP organization.** (A) Organization of the trimeric, RNA-free LASV NP. (B) The N-terminal domain of LASV NP colored by electrostatic surface potential and the C-terminal domain modeled as a cartoon (green) demonstrate the “closed” form of the NP structure. In this conformation, the RNA-binding crevice is not available to accept ssRNA. (C) To bind the viral genome, the C-terminal domain must shift away from the RNA-binding crevice to allow RNA to enter. This could be initiated by binding of NP by an as-yet-identified cofactor or perhaps the viral genome itself. (D) When bound to ssRNA, the trimer of NP will not form. Instead monomers of NP line the ssRNA backbone. Each N-terminal domain of NP interacts with the adjacent C-terminal domain of a neighboring NP.

We used X-ray crystallography and Deuterium Exchange Mass Spectrometry (DXMS), to compare the RNA-free and RNA-containing structures of Lassa NP. This analysis suggests that RNA binding is controlled through a gating mechanism of conformational changes within the N-terminal domain (Figure 2). These experiments further suggest that the trimeric form of NP stabilizes an RNA-free conformation. Upon RNA binding, the C-terminal domain of NP likely rotates slightly away from its position in the RNA-free trimer, allowing helices  $\alpha 5$  and  $\alpha 6$  to open away from the RNA-binding crevice (Figure 1). With RNA bound, NP will no longer be able to form the trimeric ring and instead may form another arrangement, such as a linear chain of NP molecules that line the ssRNA, with NP-NP interactions mediated through the N-terminal portion of one NP and the C-terminal portion of another. Such an assembly would create a continuous head-to-tail polymer attached to the RNA, in which only the RNA bound within the crevice of the N-terminal domain is resistant to RNase attack.

The tight sequestering of RNA and likely conformational change needed for replication and transcription makes NP an excellent target for small molecules that either prevent NP binding of RNA or that stabilize the RNP to prevent conformational changes induced by the polymerase. The NP of Lassa virus is well-conserved with other members of its vast arenavirus family (which includes the causative agents of Bolivian and Argentine hemorrhagic fevers, as well as lymphocytic choriomeningitis virus). Importantly, each residue that makes contact with the RNA in the Lassa NP structure is 100% conserved across the arenavirus family. Thus, the published work provides a three-dimensional template by which a path may be found to a defense against a number of pathogens that threaten human health.



**Figure 3.** Members of the Viral Hemorrhagic Fever Consortium at our Lassa virus ward and field site in Sierra Leone. The crystallographers who performed the work at SSRL are in the front row (Sapphire, left and Hastie, right).

This work was supported by the Viral Hemorrhagic Fever Research Consortium (Figure 3) and contract HHSN272200900049C (BAA-NIAID-DAIT-NIHAI2008031, EOS and VJW), grants AI077719 (JCT), AI047140 (JCT), GM093325 (VJW), RR029388 (VJW), an Investigators in Pathogenesis of Infectious Diseases award from the Burroughs Wellcome Fund (EOS), and the Skaggs Institute for Chemical Biology (EOS).

## References

1. K. M. Hastie, C. R. Kimberlin, M. A. Zandonatti, I. J. MacRae & E. O. Saphire, *Proc Natl Acad Sci U S A* **108**, 2396-2401, (2011).
2. X. Qi, *et al. Nature* **468**, 779-783, (2010).
3. K. M. Hastie, L. B. King, M. A. Zandonatti & E. O. Saphire, *PLoS one* **7**, e44211, (2012).

## Primary Citation

K. M. Hastie, T. Liu, S. Li, L. B. King, N. Ngo, M. A. Zandonatti, V. L. Woods, Jr., J. C. de la Torre, E. O. Saphire, Crystal Structure of the Lassa Virus Nucleoprotein–RNA Complex Reveals a Gating Mechanism for RNA Binding", *Proc. Natl. Acad. Sci. USA* **108**, 19365 (2011).doi: 10.1073/pnas.1108515108.

## Authors

*Kathryn Hastie and Erica Ollmann Saphire, The Scripps Research Institute*

SSRL is primarily supported by the DOE Offices of Basic Energy Sciences and Biological and Environmental Research, with additional support from the National Institutes of Health, National Institute of General Medical Sciences and the National Center for Research Resources.