

# The Role of Arbovirus UTRs on Neurotropism

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## 1. Background and outline

Emerging and re-emerging arthropod-borne viruses such as Japanese encephalitis (JEV), Dengue (DENV), Yellow fever (YFV), and Chikungunya (CHIKV) viruses are a growing global health threat. **Zika virus (ZIKV)** is a neurotropic flavivirus (FV) that can cause **congenital infection**, which can result in **microcephaly** and **fetal demise**. Recently, the translational regulator protein **Musashi-1 (Msi1)** has been attributed to **promoting ZIKV replication, neurotropism, and pathology** [1]. Msi1 predominantly binds single-stranded UAG motifs in the 3'UTR of RNA [2].

Here we systematically analyzed the thermodynamic properties of **Musashi binding elements (MBEs)** in the 3'UTR of 76 arbovirus genomes *in silico*. Our results indicate that MBEs in the ZIKV 3'UTR occur predominantly in unpaired, single-stranded structural context, thus **supporting experimental observations** of Msi1 binding affinity with a thermodynamic model of RNA structure formation.

## 2. Flavivirus 3'UTR mediates pathogenicity

Flaviviruses are small (+)ssRNA viruses of 10-12kB length with highly structured UTRs. Upon infection, accumulation of stable long non-coding viral RNA, sub-genomic flaviviral RNA (**sfRNA**), is observed. sfRNAs can modulate cellular function and are linked to pathogenicity. They are stable decay intermediates produced by partial degradation of the viral genome by 5'-3' exonuclease Xrn1, which is efficiently stalled at **evolutionary conserved xrRNA structures**.

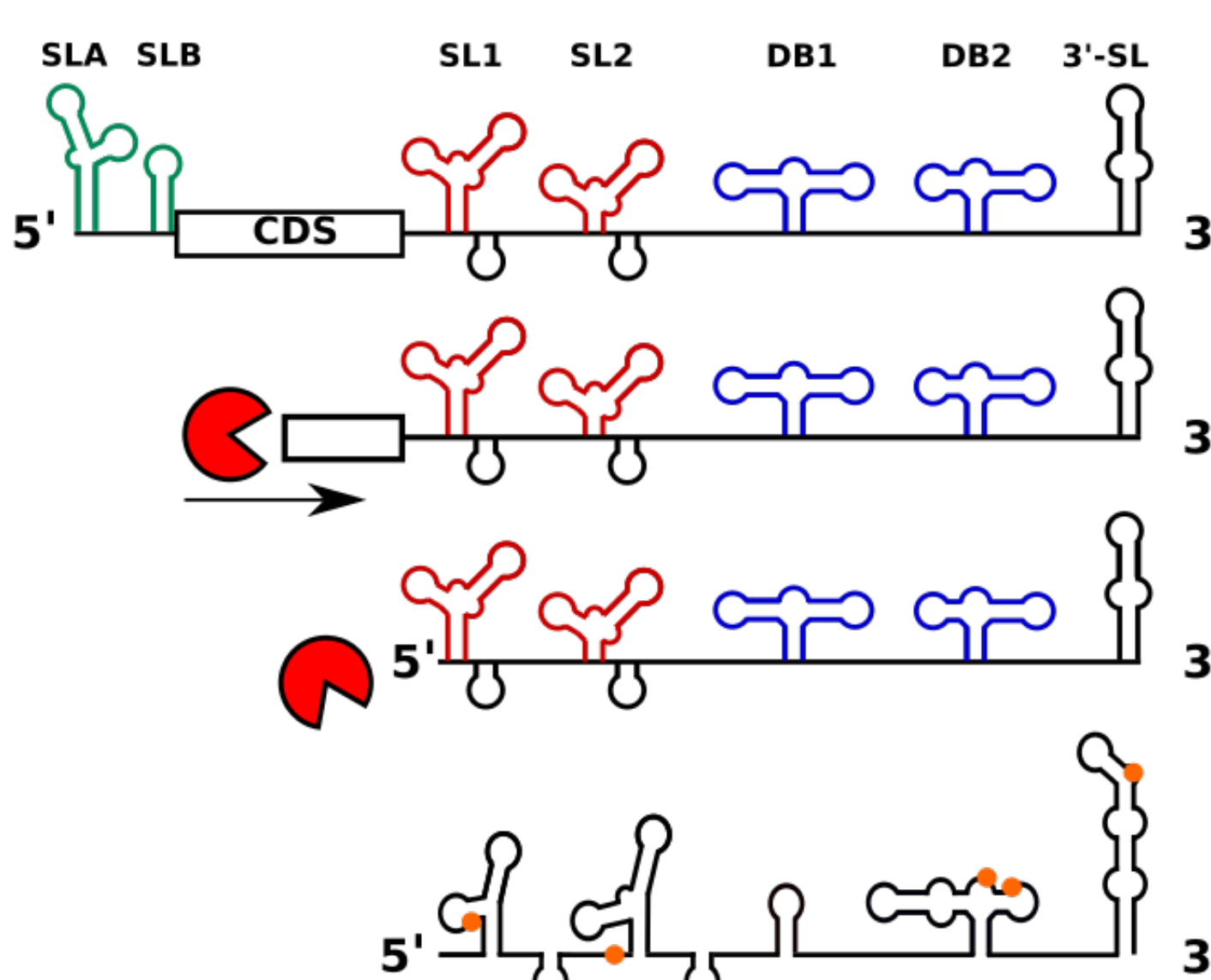


Fig 1. Top: Schematic representation of FV 3'UTR hijacking the host mRNA degradation pathway. Conserved xrRNA stem-loop (SL) and dumbbell (DB) elements are located in single or tandem copies within 3'UTRs and stall the host exonuclease Xrn1 (red pac-man). Bottom: ZIKV has two SL and one DB element. Location of UAG MBEs are highlighted in orange.

## 3. Opening energy and single-strandedness

We use the **ViennaRNA Package** [3] to model the thermodynamics of RNA secondary structure formation. The partition function  $Z$  allows for computation of the equilibrium probability of secondary structure  $s$

$$Z = \sum_s e^{-E(s)/RT} \quad P(s) = \frac{e^{-E(s)/RT}}{Z}$$

The accessibility (i.e., the probability that a region  $i \dots j$  along the RNA is single-stranded) can be derived from  $Z$ . The opening energy (i.e., the free energy required to force the region to be in a single-stranded structural context) is computed as  $\Delta G_{\text{open}} = -RT \ln P(\text{unpaired})$ .

Low opening energy indicates single-strandedness. We compute local pairing probabilities of trinucleotides to assess the likelihood of MBE single-strandedness in a genomic context. Comparison to a large sample of randomized sequences allows computing a  $z$  score for each trinucleotide.

## 4. MBE accessibility in ZIKV 3'UTR

We analyzed the accessibility of all trinucleotides in the coding region (CDS) and 3'UTR of ZIKV from Brazil and found a marked difference in the distribution of  $z$  scores, suggesting different sequence composition. Musashi-binding **UAG trinucleotides are maximally accessible** in the 3'UTR, which **corroborates experimental studies** [1,4].

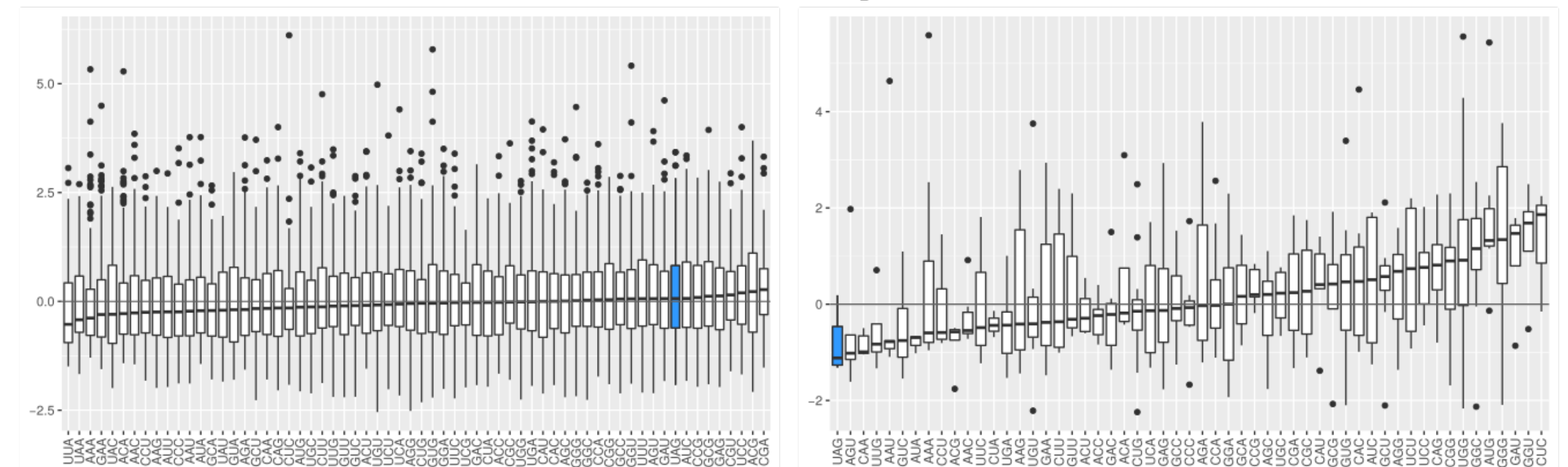


Fig 2. Distribution of  $z$  scores for all trinucleotides in the CDS (left) and 3'UTR (right) of ZIKV-BR, sorted by median  $z$  score. Interquartile ranges are homogeneous within the CDS region. The MBE motif UAG (blue) is maximally accessible in the 3'UTR.

## 5. MBE accessibility in the 3'UTR of flaviviruses and alphaviruses

To address the broader question whether other viruses have a similar neurotropic potential to ZIKV in the developing fetus, we analyzed MBEs in the 3'UTR of related arbovirus genomes. Below, we show the distribution of MBE opening energy  $z$  scores for flaviviruses (left) and alphaviruses (right). Low overall  $z$  scores indicate unpaired UAG motifs, suggesting **high Msi1 affinity**. We use this as an **estimator for teratogenicity**.

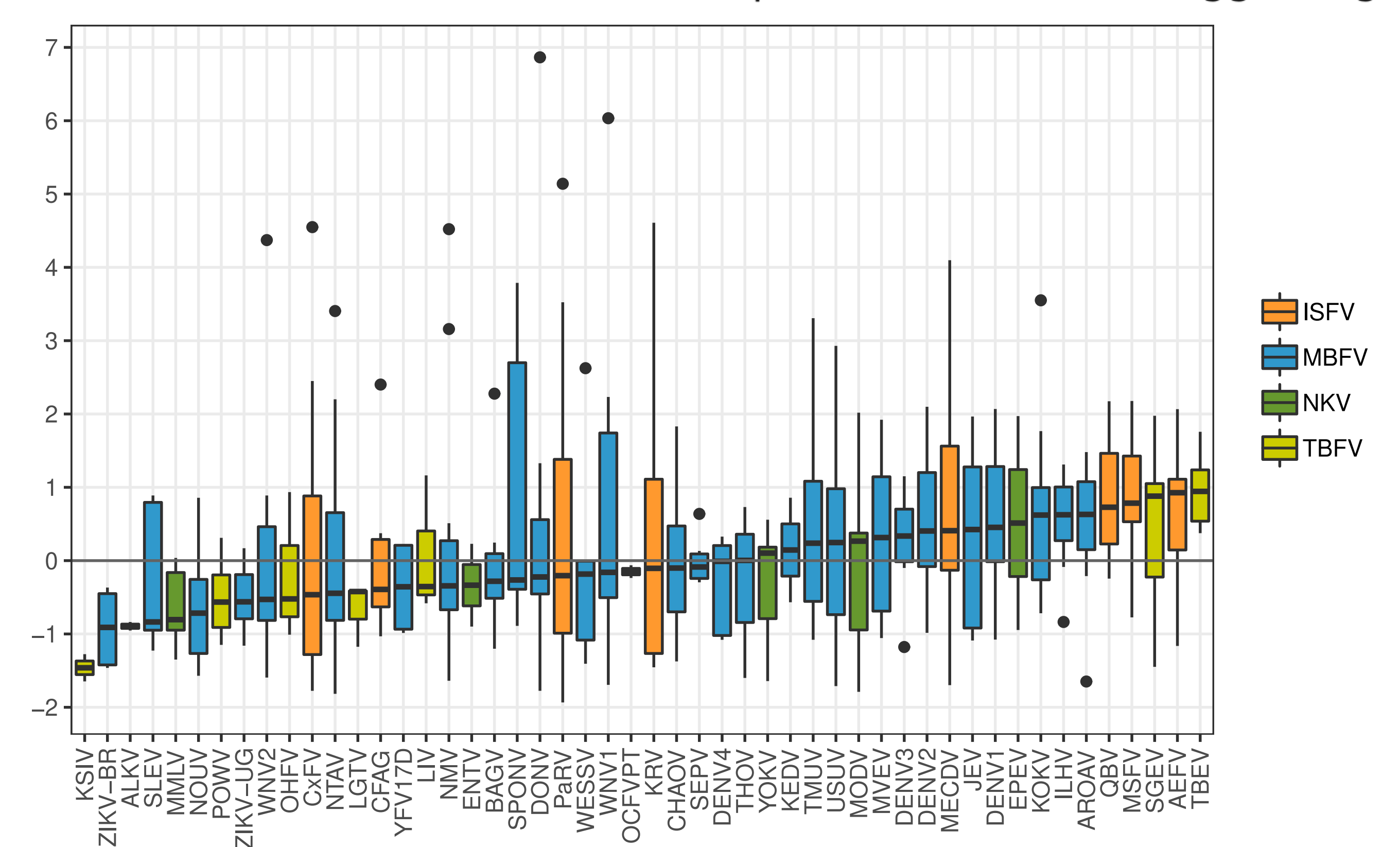


Fig 3. Distribution of MBE opening energy  $z$  scores in flavivirus 3'UTRs. Colors indicate serocomplexes (ISFV: Insect-specific flaviviruses; MBFV: Mosquito-borne flaviviruses; NKV: No known vector flaviviruses; TBFV: Tick-borne flaviviruses). Only viruses with more than two MBEs within the 3'UTR shown.

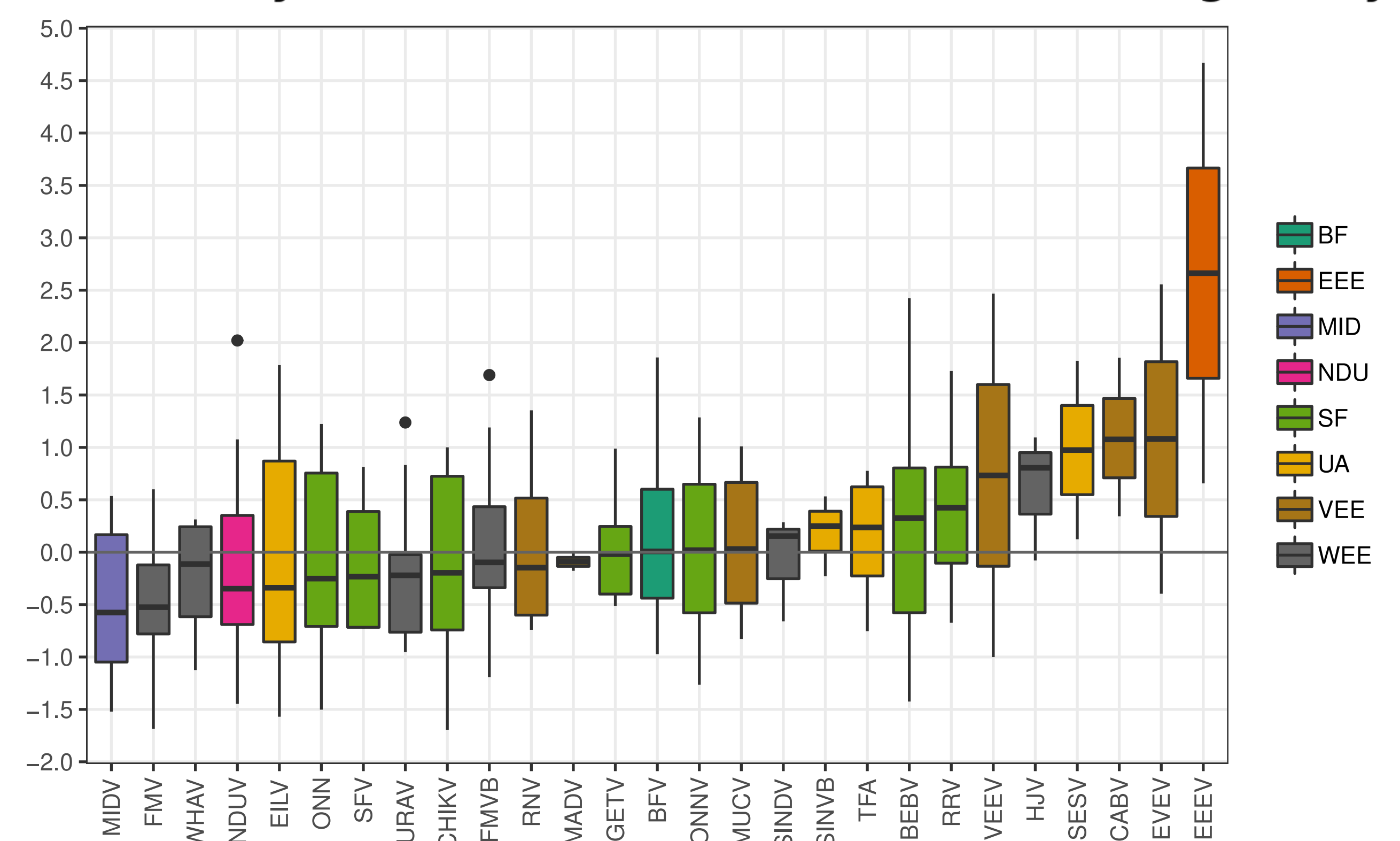


Fig 4. Distribution of MBE opening energy  $z$  score in alphavirus 3'UTRs. (BF: Barmah virus complex; EEE: Eastern equine encephalitis complex; MID: Middelburg virus complex; NDU: Ndumu virus complex; SF: Semliki forest complex; VEE: Venezuelan equine encephalitis complex; WEE: Western equine encephalitis complex; UA: Unassigned alphaviruses).

Among flaviviruses, the **Brazilian ZIKV has the lowest median MBE opening energy  $z$  scores**, followed by the neurotropic viruses SLEV, WNV, and POWV, which can cause transplacental infection, severe neuropathology and fetal demise [5]. Neurotropic alphaviruses such as SINDV, VEEV and EEEV, and the teratogenic SFV do not show a strictly negative  $z$  score distribution, as seen for some flaviviruses.

We employed an established biophysical model of RNA structure formation to analyze the thermodynamic properties of MBEs *in silico*. Our results underline experimental studies suggesting that **ZIKV is not alone in its capacity to cause severe neuropathology** [6]. The tropism of other arboviruses might have been overseen due to insufficient epidemiologic data.

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