

Explaining Flavivirus Congenital Neurotropism with Thermodynamics

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Flaviviruses are a Global Health Threat

- FV include human pathogens like YFV, DENV, JEV, WNV, TBEV
- Typically transmitted by mosquitoes and ticks
- Millions of infections every year
- Broad range of clinical manifestations
 - Headache / rash / (hemorrhagic) fever / meningitis / encephalitis
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- 2015-2017 ZIKV outbreak in the Americas
- Congenital neurotropism
- High increase in microcephaly cases in newborns



Source: Wikipedia

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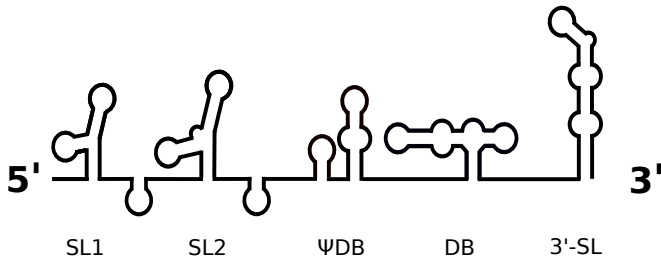


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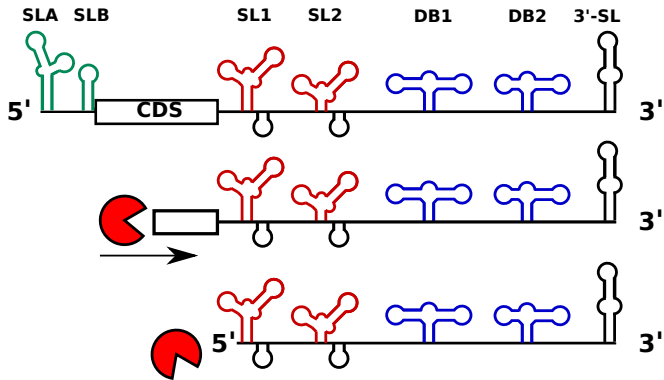
Are there other emerging FV that have a similar neurotropic potential in the developing fetus?

Flavivirus genome characteristics

- Single-stranded, positive-strand RNA viruses
- Enters cell through receptor-mediated endocytosis
- Capped, non-polyadenylated genome (gRNA) of 10-12kb length
- Encodes a single ORF, flanked by structured 5'-UTR and 3'-UTR
- Translation of FV ORF yields a single polyprotein



Flaviviruses hijack the host mRNA degradation machinery



- Accumulation of short flavivirus RNA (sfRNA) upon infection
- Stable decay intermediates produced by partial Xrn1 degradation
- Xrn1 is efficiently stalled at conserved xrRNA structures
- sfRNA modulates pathogenicity
- Many host proteins bind sfRNA

Musashi interacts with 3'UTR

Musashi (Msi) is a highly conserved family of proteins in vertebrates and invertebrates that act as a translational regulator of target mRNA.

- Involved in cell proliferation and differentiation
- Two paralogs in mammals, Msi-1 and Msi-2
- Expressed in stem cells and overexpressed in tumors
- Two RNA-regulation domains (RRM)
- Binds single-stranded UAG core motif in the 3'UTR of mRNA

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A Conserved Three-nucleotide Core Motif Defines Musashi RNA Binding Specificity*

Received for publication, July 16, 2014, and in revised form, October 20, 2014. Published, JBC Papers in Press, November 3, 2014, DOI 10.1074/jbc.M114.597112

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Mol Cell Biol 35:2965-2978 (2015)

RNA-Binding Protein Musashi1 Is a Central Regulator of Adhesion Pathways in Glioblastoma

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Does Msi interact with flavivirus 3'UTR?

Musashi is involved in ZIKV neurotropism

A recent study has shown that Msi-1 promotes ZIKV replication in glioblastoma, neuroblastoma and neuronal stem cells

Science

REPORTS

Cite as: P. L. Chavali *et al.*, *Science*
10.1126/science.aam9243 (2017).

Neurodevelopmental protein Musashi 1 interacts with the Zika genome and promotes viral replication

Pavithra L. Chavali,^{1*†} Lovorka Stojic,^{1*} Luke W. Meredith,² Nimesh Joseph,¹ Michael S. Nahorski,³ Thomas J. Sanford,² Trevor R. Sweeney,² Ben A. Krishna,⁴ Myra Hosmillo,² Andrew E. Firth,² Richard Bayliss,⁵ Carlo L. Marcelis,⁶ Susan Lindsay,⁷ Ian Goodfellow,² C. Geoffrey Woods,³ Fanni Gergely^{1‡}

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Another study addressed the question whether related arboviruses could cause transplacental infection and fetal demise

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

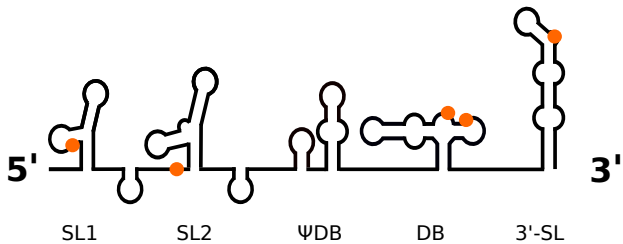
EMERGING INFECTIONS

Zika virus–related neurotropic flaviviruses infect human placental explants and cause fetal demise in mice

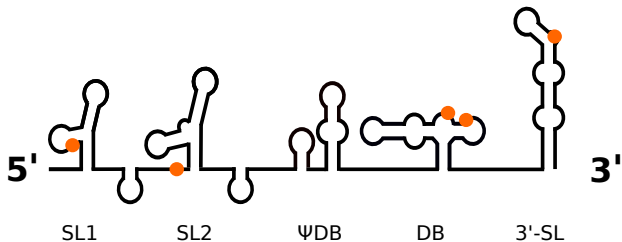
Derek J. Platt,^{1*} Amber M. Smith,^{2*} Nitin Arora,^{3,4} Michael S. Diamond,^{1,2,5,6}
Carolyn B. Coyne,^{3,4} Jonathan J. Miner^{1,2,5†}

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ZIKV 3'UTR revisited



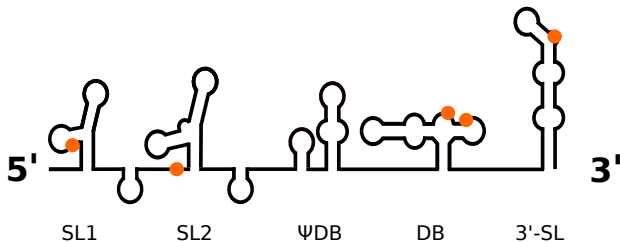
ZIKV 3'UTR revisited



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

$$\Delta G_{\text{open}} = -RT \ln P(\text{unpaired})$$

ZIKV 3'UTR revisited

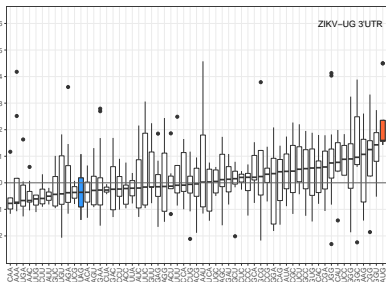
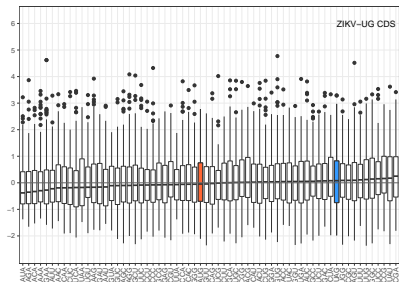
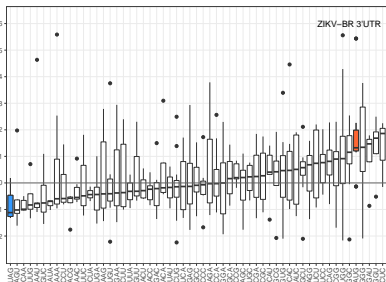
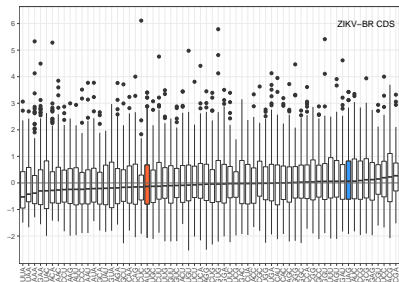


$$Z = \sum_s e^{-E(s)/RT}$$

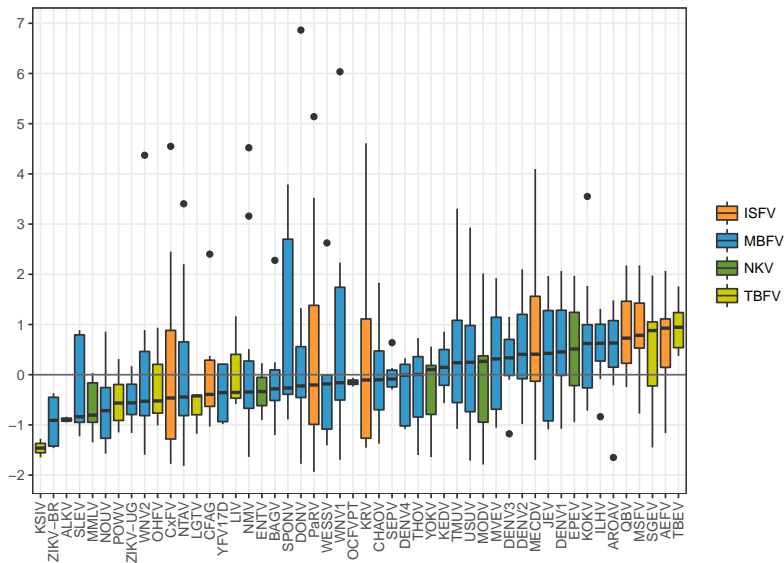
$$\Delta G_{\text{open}} = -RT \ln P(\text{unpaired})$$

We use `RNAplfold` to compute the opening energy of trinucleotide x in a genomic (± 100 nt) as well as a shuffled sequence context and compute a z score statistics.

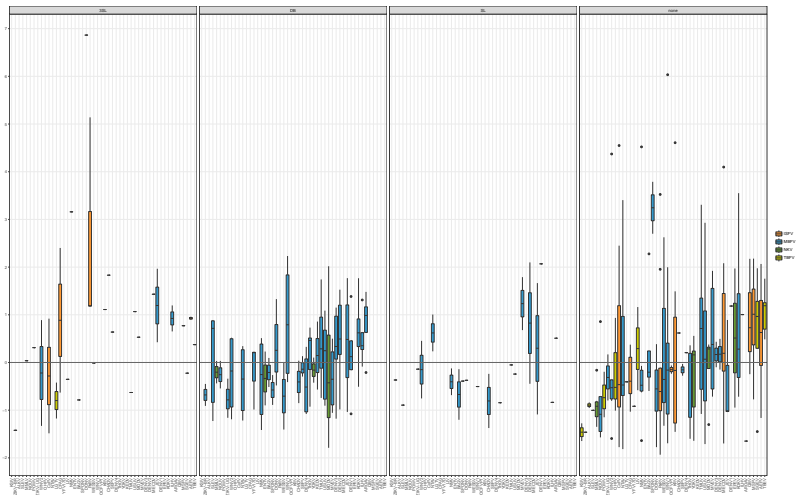
Trinucleotide opening energies in ZIKV



MBE opening energies in related viruses

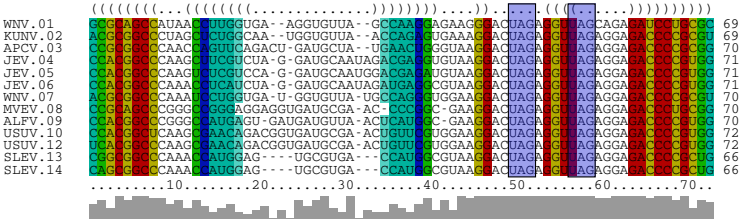
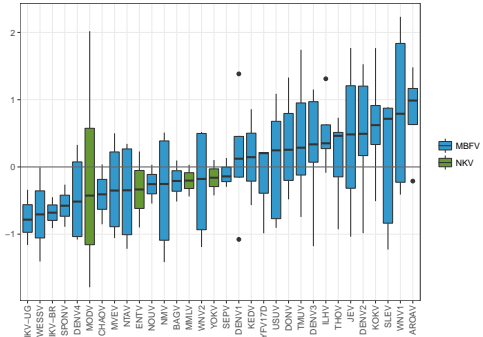


MBEs opening energies by xrRNA



MBEs accumulate within dumbbell (DB) elements in MBFV and NKV

MBE in MBFV and NKV DB elements



Structure and sequence conservation in DB elements

```

(((((((((((.....((((((((.....)))))))))))))).)))))).....((((((((.....))))))))))
YORK_NC_005039.1 DB.1 211-292 | GGGAGCCUCCGCC --- AAUGGGGUUUA CAUUAUUGAGCUCGCAUUGGUCUAGGGGAC UAGCGGUUAGAGGAGACCUCUCC 82
DONY_NC_010997.1 DB.1 140-216 | AGGGGCGCAGUDC --- UGUGUCUCUCA --- AUCUCAGGACACUUGACUCUAGCGC --- UGCGAC UAGCGGUUAGAGGAAACCCGCCA 77
CHAOV_NC_017086.1 DB.1 130-201 | AGGGGGCACACUGC - C-ACCAGC --- AGCCCGAGUCUGCAACGGCA - UG --- UGUACU GCGGCUUAGAGGAGACCCCGCCA 72
SEPV_NC_008719.1 DB.1 262-333 | AGGGAGGCAUCUCC --- CUGUCGC --- GUCCGCGCCAG - AGGAAAGAAAGGGGUU UAGAGGUUAGAGGAGACCUCUCC 72
WEVW_NC_012735.1 DB.1 274-345 | UGGGAGCCUCCAC - CCACCC --- UAUCGCGCCAGGGU - GGGAAAGUAGGGGUU UAGAGGUUAGAGGAGACCACUUC 72
YEFF17D_NC_020203.1 DB.1 1307-379 | CCAGGAGCCUCCGC --- UACCACCC --- UCCACCGUGUGUG --- UAGAAGACCGGGGUC --- UGGAAGGC - AC UAGAGGUUAGAGGAGACCUCUCC 73
USUV_NC_009942.1 DB.1 340-409 | CCAGGCGCCCAAGC --- GACACACG --- GUUGUGGACUGUG --- UCG --- UGGAAGGC - AC UAGAGGUUAGAGGAGACCUCUCC 72
MVEV_NC_000943.1 DB.1 340-409 | CCAGGCGCCCGGGC --- CGGAGGAG --- GUUGUGCAAAC - CG --- CG - G- GAAGG - AC UAGAGGUUAGAGGAGACCUCUCC 70
JEV_NC_001437.1 DB.1 310-380 | CACCGGCCECAAGC --- CUCUGUAG --- GAUGCAAU - AGAGC --- AGG --- UGUAAGG - AC UAGAGGUUAGAGGAGACCUCUCC 71
AROV_NC_009026.2 DB.1 141-211 | GGCGCCCGCAAGC --- CUGGUAUG --- AACCGUUG - AUCC - AGG --- CGAAGGG - AC UAGAGGUUAGAGGAGACCUCUCC 71
TMUV_NC_015843.2 DB.1 348-416 | CCAGGCGCUGAAA --- UGAUGAGC --- U - GGUGUGUACU --- CAU --- GGAAGG --- AC UAGAGGUUAGAGGAGACCUCUCC 69
ZIKV_BR_NC_035889.1 DB.1 238-307 | UGGGGGCGUAGAC --- UGGALUCA --- G --- CUGUGAUCUC --- CAG --- AAGAGGG --- AC UAGUGUAGAGGAGACCUCUCC 70
ZIKV_UG_NC_012532.1 DB.1 238-307 | CUGGGGCGUAGAC --- UGGALUCA --- G --- CUGUGAUCUC --- CAG --- CAGAGGG --- AC UAGUGUAGAGGAGACCUCUCC 70
SPONV_SA_Ar DB.1 206-274 | UGGGGGCGUAGAC --- GCAGAGGA --- G --- CUGUGACUCCU --- GCU --- GGAAGG --- AC UAGUGUAGAGGAGACCUCUCC 69
NMV_NC_032088.1 DB.2 364-428 | UGUUGGCGCC - AAC --- CGAG - U - G --- AAGUCU - AAA - CU --- CGU --- GGAAGG --- AC UAGAGGUUAGAGGAGACCUCUCC 65
NTAV_NC_018705.3 DB.2 441-505 | GC AAGCGCC - AAG --- UUGG - C - A --- AAGUCU - AAA - CC --- AGG --- GGAAGG --- AC UAGAGGUUAGAGGAGACCUCUCC 65
WNV1_NC_009942.2 DB.2 438-503 | GCAGGCGCC - AGC --- CUGG - CU - G --- AAGUCU - AGG - UC --- AGG --- GGAAGG --- AC UAGAGGUUAGAGGAGACCUCUCC 66
WNV2_NC_001563.2 DB.2 382-447 | CCAGGCGCC - AAC --- UUGG - CU - G --- AAGUCU - AAG - C --- AGG --- GGAAGG --- AC UAGAGGUUAGAGGAGACCUCUCC 66
MVEV_NC_000943.1 DB.2 417-483 | GAGUGGCGC - AAG --- CUGC - CG - G --- AAGUCUAGAG - CG --- GGU --- GGAAGG --- AC UAGAGGUUAGAGGAGACCUCUCC 67
SLEV_NC_007580.2 DB.2 359-426 | C AAGGCGCCAAAC - CGC - CUCA --- AAGUCUAGAG - AC --- GGG --- GGAAGG --- AC UAGAGGUUAGAGGAGACCUCUCC 68
DENV4_NC_002640.1 DB.2 203-269 | AGGGGCGCCAGAG - CAG - GAGG --- AAGUCUAUC - CU --- GGU --- GGAAGG --- AC UAGAGGUUAGAGGAGACCUCUCC 69
DENV2_NC_001474.2 DB.2 268-335 | UGGGGGCGCC - AAG --- GCGA - GAUG --- AAGUCUAGUC - UC --- GCU --- GGAAGG --- AC UAGAGGUUAGAGGAGACCUCUCC 68
TROV_NC_04151.1 DB.2 359-426 | CC AAGCGCC - AAU --- UCUC - GUUG --- AAGUCUGAC - CA - GAG --- GGAAGG --- AC UAGAGGUUAGAGGAGACCUCUCC 68
DENV1_NC_001477.1 DB.2 278-345 | GCGGGGCGCC - AAC --- ACCA - GGGG --- AAGUCUAGCC - UG --- UUG --- UUAAGG --- AC UAGAGGUUAGAGGAGACCUCUCC 68
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MLV_NC_0004119.1 DB.1 200-251 | GGGAGGC - C --- CGAAGC --- AAAGCUA --- AAGAGG --- AAGG --- AC UAGCGGUUAGAGGAGACCUCUCC 52

```

- Highly conserved MBE pair in DB elements
- Central multiloop and distal hairpin loop

- Our biophysical model corroborates experimental findings of Msi involvement in ZIKV replication
- MBEs in flavivirus DB elements show primary sequence conservation
- ZIKV might not be alone in its capacity to cause severe neuropathology in the developing fetus
- A possible role of Msi in the replication cycle of some FV?

Acknowledgments

Collaborator

Adriano De Bernardi Schneider
(UNC Charlotte)

TBI Vienna

Ivo L. Hofacker
Roman Ochsenreiter
Andrea Tanzer

FWF

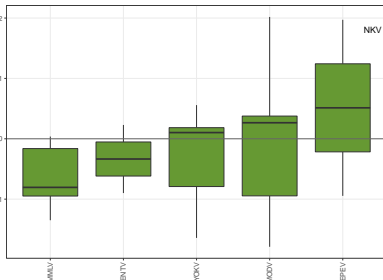
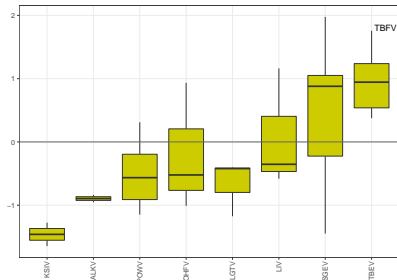
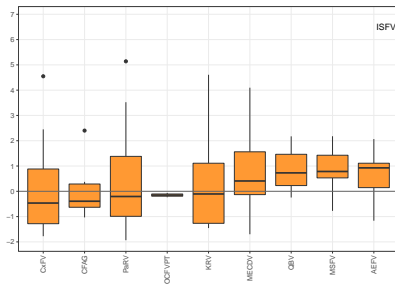
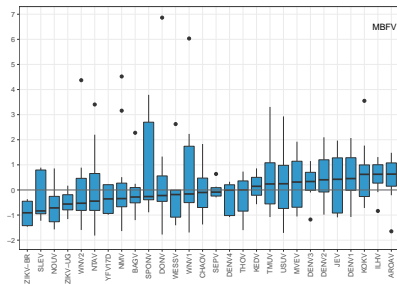
Der Wissenschaftsfonds.

SFB RNA regulation of the
transcriptome (FWF-F43)



Thank you!

MBE opening energies in related viruses



MBE opening energies in related viruses

