Flexible RNA-RNA Interactions in Influenza A Virus

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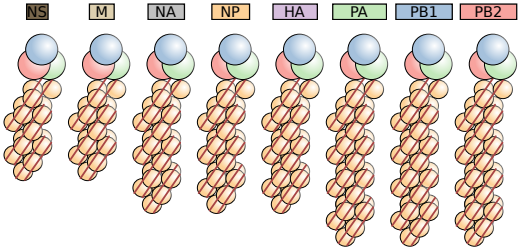
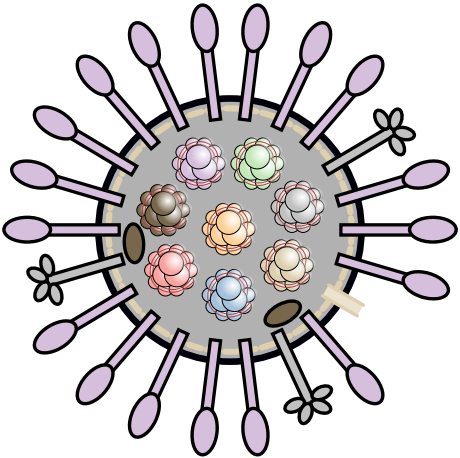
February 12, 2020

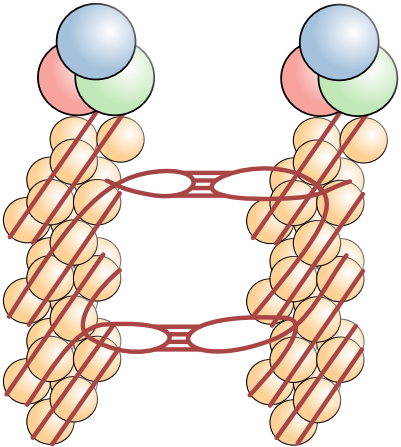
Influenza A Virus (IAV): Selective '7+1' Segment Packaging





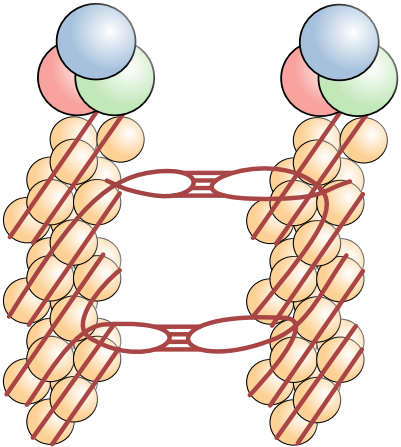
[Eisfeld et al., 2015]

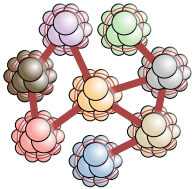
Inter Segment RNA-RNA Interactions: Pairing of IAV Segments



[Fournier et al., 2012]

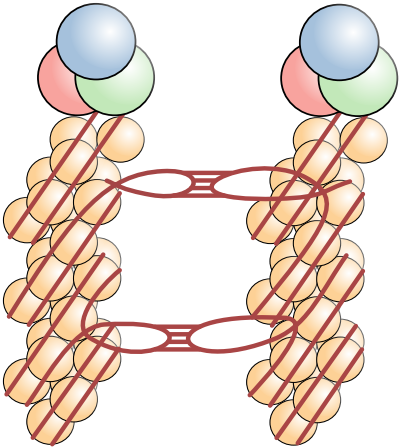
Inter Segment RNA-RNA Interactions: Pairing of IAV Segments

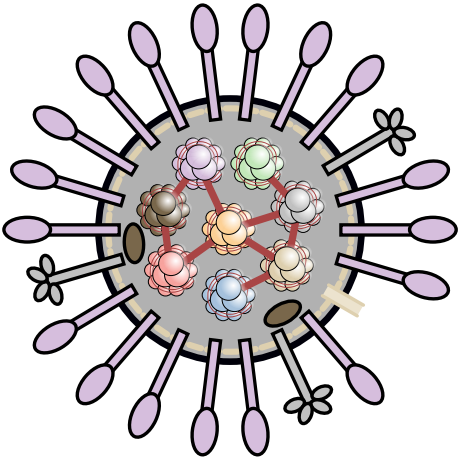




[Fournier et al., 2012]

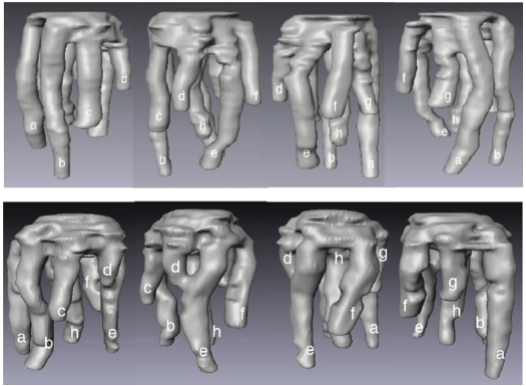
Inter Segment RNA-RNA Interactions: Pairing of IAV Segments

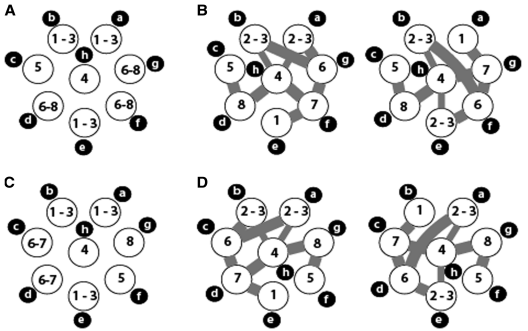




[Fournier et al., 2012]

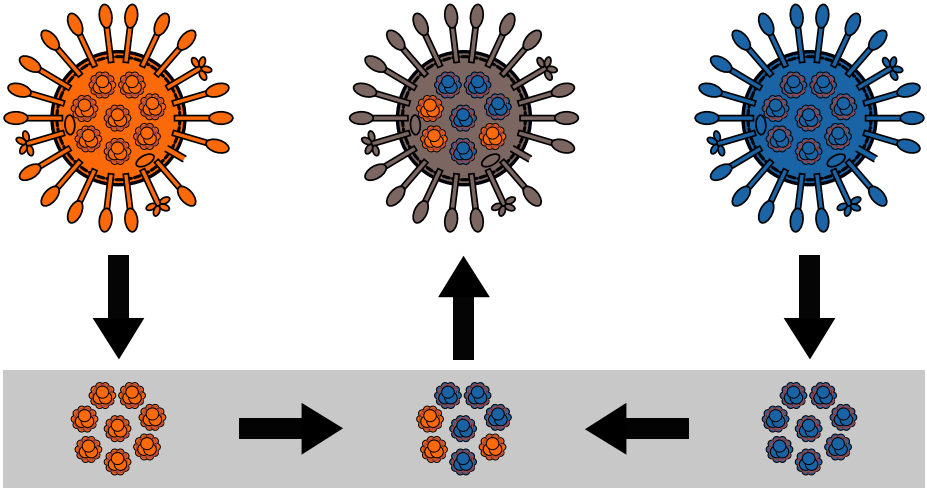
Electron Tomography: Variable Packaging of IAV Segments





[Fournier et al., 2012]

Reassortment: Packaging of Multiple Strains

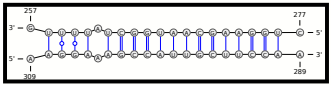


[Li et al., 2010]

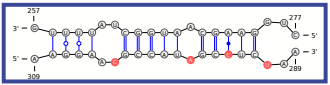
In Vitro Interaction: Avian H5N2, PB1 and NS Segment

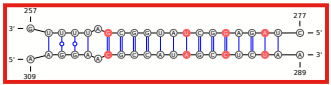
Mutation Experiment

-26.70 kcal/mol

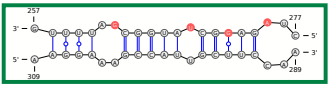


-14.20 kcal/mol





-28.50 kcal/mol



-16.10 kcal/mol

[Gavazzi et al., 2013]

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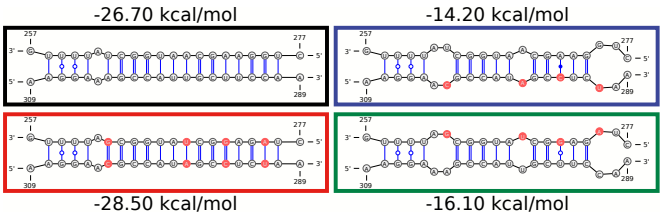
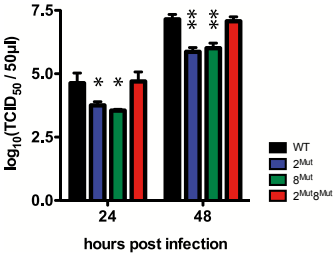
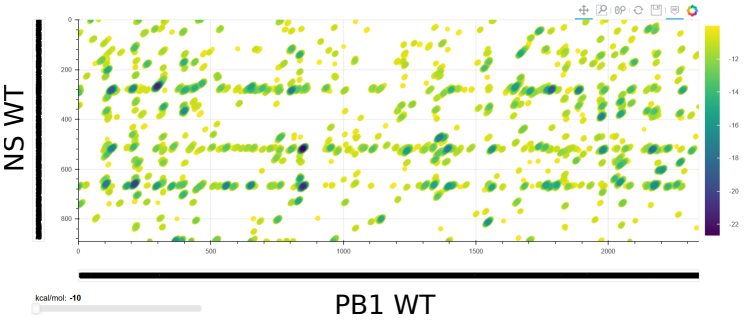


Viral Titer Estimation



[Gavazzi et al., 2013]

vRNAsite: Identified Hot Spots on NS Segment



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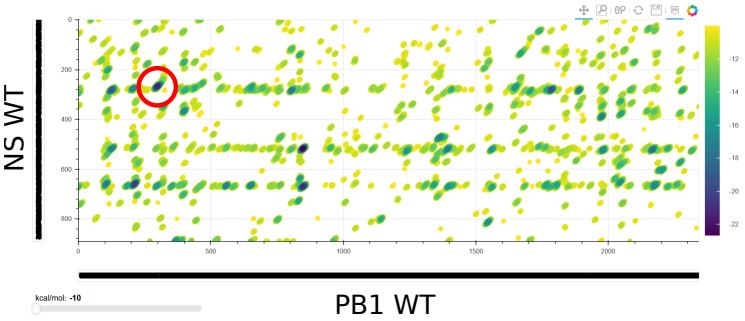

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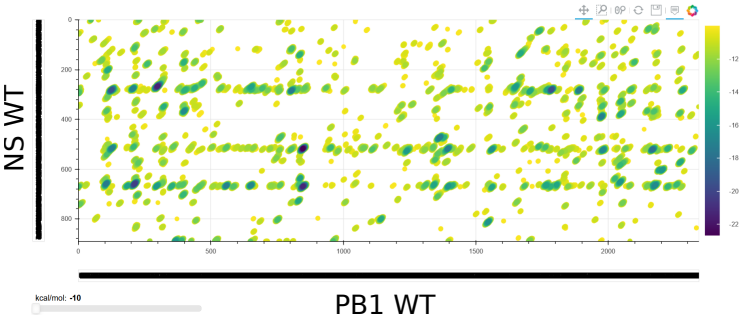

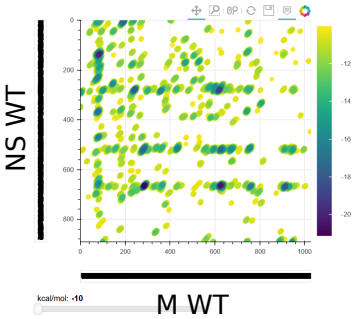

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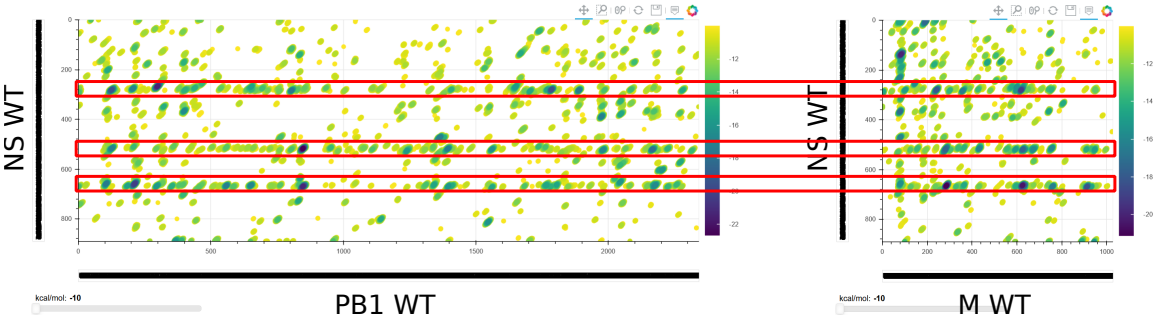

Scoring Function for Flexible Regions

RNAsubopt Threshold

$\delta_{sub} = -percentage \cdot top_{energy}$

[Lorenz et al., 2011]

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Flexibility Score

$$flex_{score} = \frac{sub_{energy}}{top_{energy}} \cdot \frac{bp_{distance}(sub_{structure}, top_{structure})}{sequence_{length}}$$

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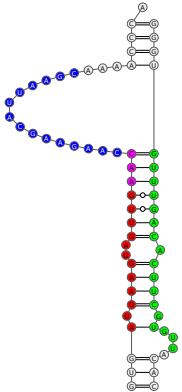
structure	$energy$	bp_{dist}	$flex_{score}$
((((.((((..(((((((.................((((.&))))))))))).)))))...))))..	-15.80	0	0.0000
.............(((((...((((..(((((((...((((.&))))))))))..)))))..))).)).	-15.30	30	0.4374
.............(((((...((((..(((((((...((((.&))))))))))..)))))..))).)).	-15.20	32	0.4635
((((.((((..(((((((.................((((.&))))))))))).)))).)..))))..	-15.10	2	0.0288
..............(((...((((..(((((((...((((.&))))))))))..)))))..))))....	-15.00	28	0.4002
..............(((...((((..(((((((...((((.&))))))))))..)))))..))))....	-14.90	30	0.4260

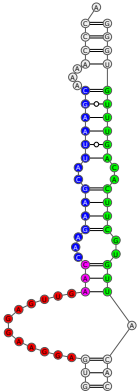
[Lorenz et al., 2011]

Flexible IAV Segment Interaction

Dadonaite et al.



Constrained Structure



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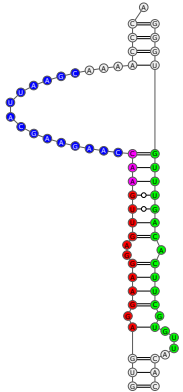

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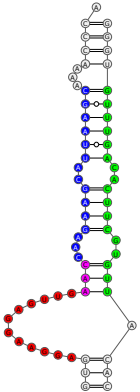
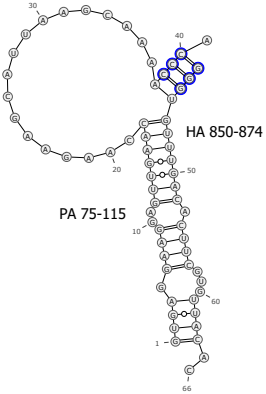
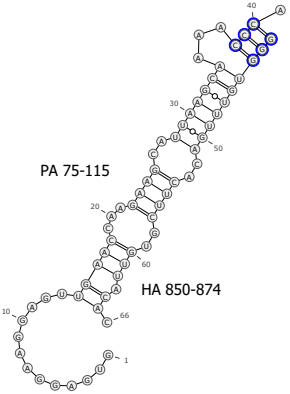
Constrained Structure



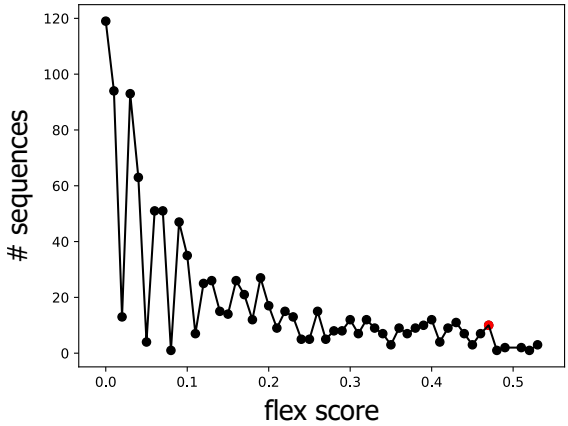
Top Structure
-15.80 kcal/mol

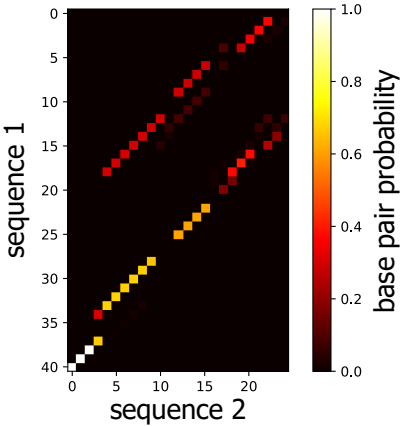


Sub Structure
-15.20 kcal/mol
score: 0.4664



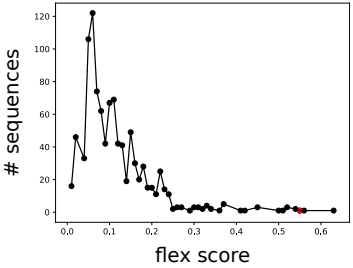
Dinucleotide Shuffling Distribution



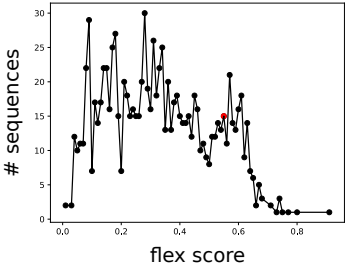


1000x Score Sampling of Functional RNAs

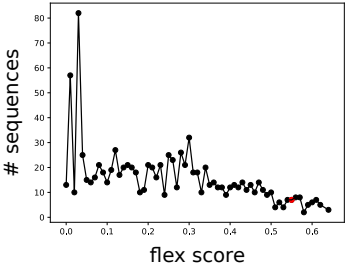
microRNA 154



Small nucleolar RNA17

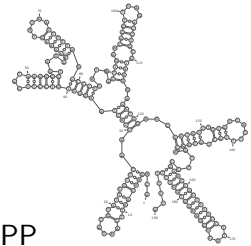


tRNA

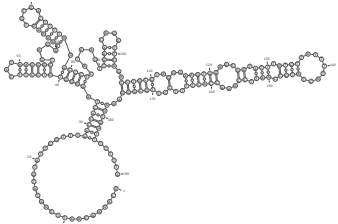


TPP Riboswitch: Problems with Complex Structures

With TPP



Without TPP



[Mironov et al., 2019]

TPP Riboswitch: Problems with Complex Structures

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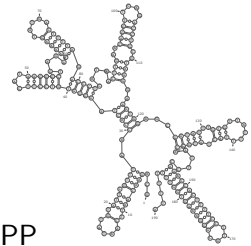
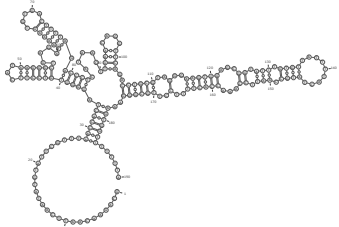
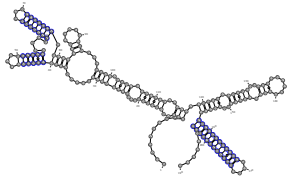


Without TPP

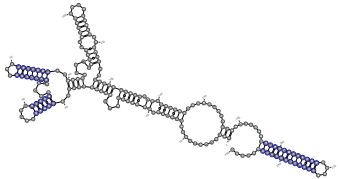


[Mironov et al., 2019]

Top Structure -50.90 kcal/mol

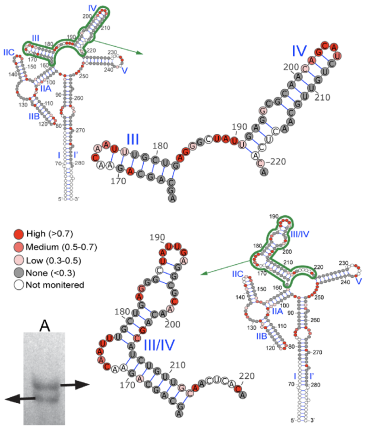


Sub Structure -47.90 kcal/mol
score: 0.3616



HIV-1 Rev Response Element: Focus on Flexible Part

Sherpa et al.



[Sherpa et al., 2019]

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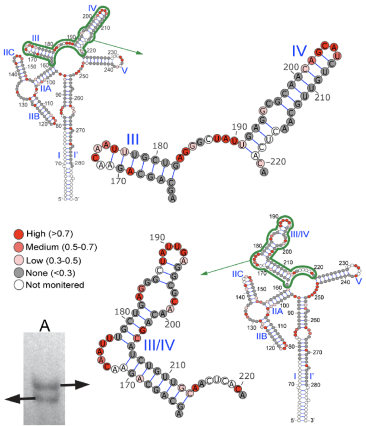
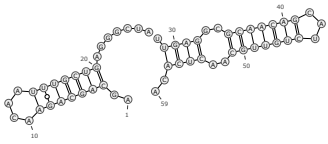
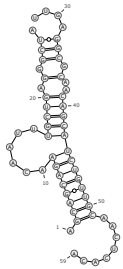


[Sherpa et al., 2019]

Sub Structure -21.70 kcal/mol
score: 0.5696



Top Structure -22.60 kcal/mol



Acknowledgements

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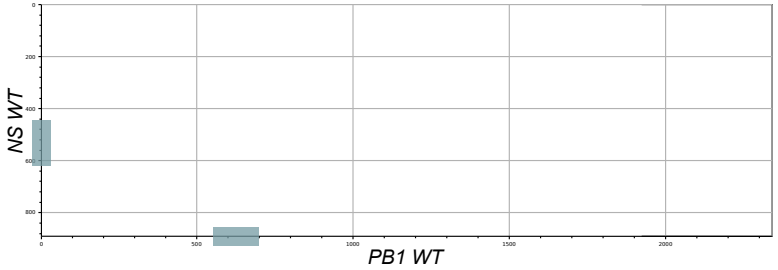
Cell, 111:747–756.

vRNAsite

input

- fasta
- IAV segments
- mutation sites

- sliding window across two segments



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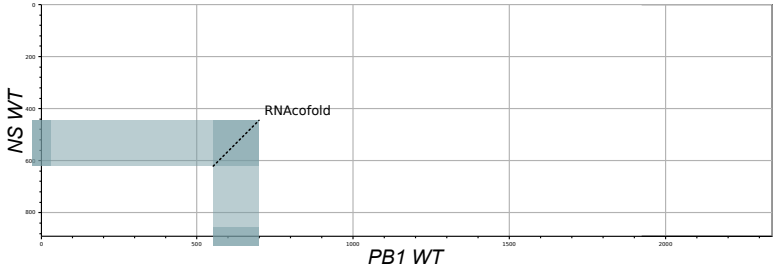
method

- sliding window across two segments
- calculate MFE with RNAcofold



[Lorenz et al., 2011]

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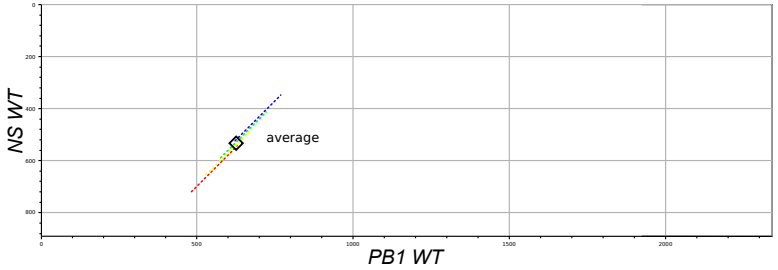

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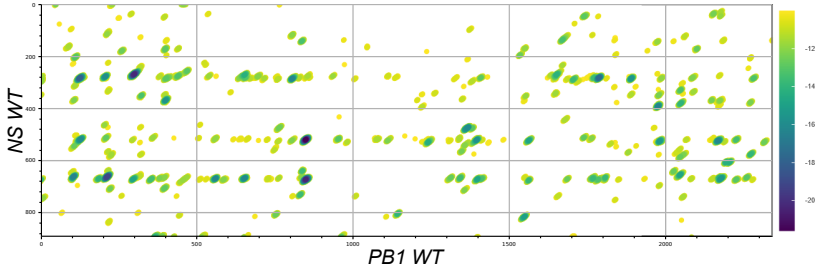
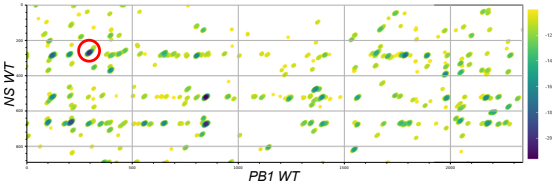
- calculate MFE with RNAcofold
- take average for every base pairing

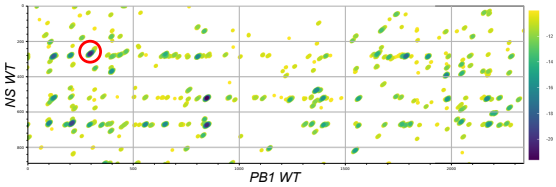


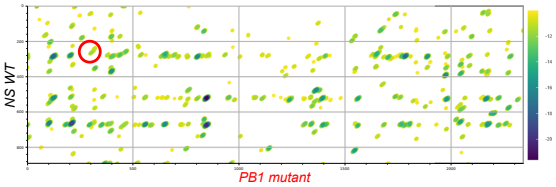
[Lorenz et al., 2011]

vRNAsite: In Vitro Interaction

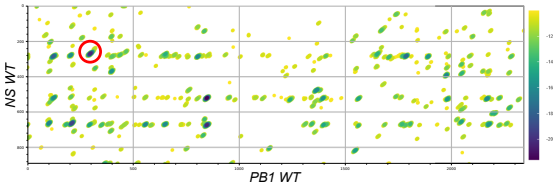


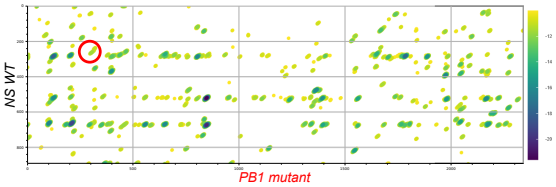
vRNAsite: In Vitro Interaction

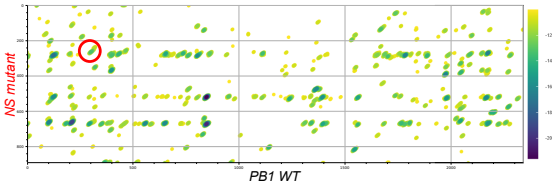




vRNAsite: In Vitro Interaction







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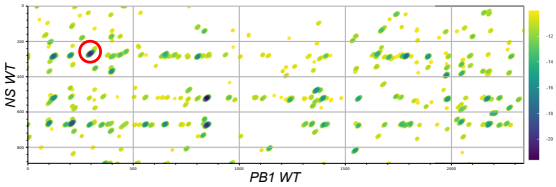
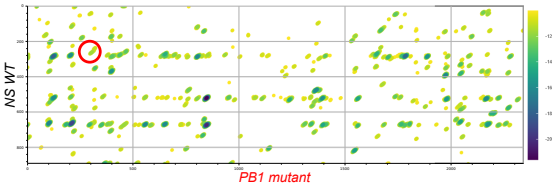
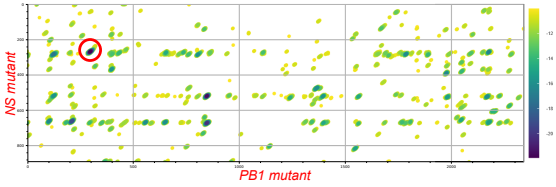
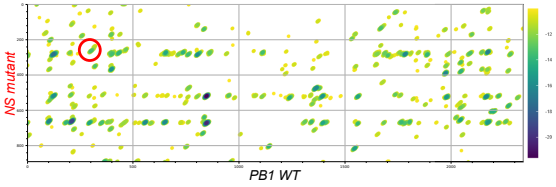
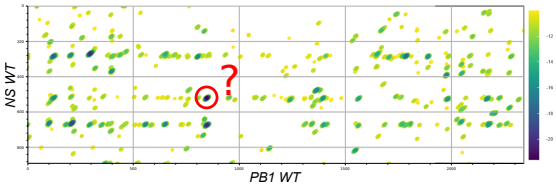


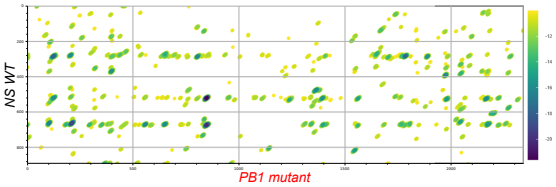


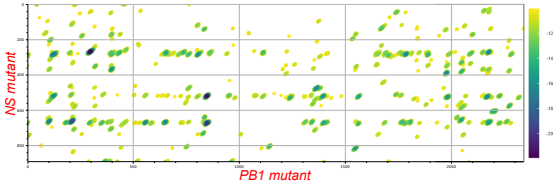


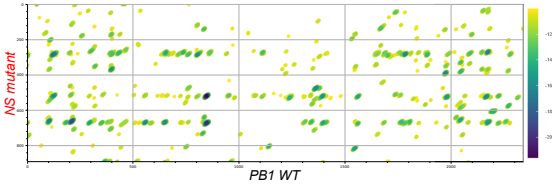


vRNAsite: In Vitro Interaction



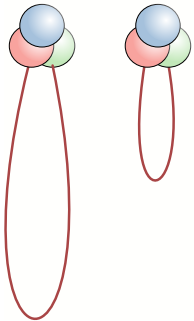






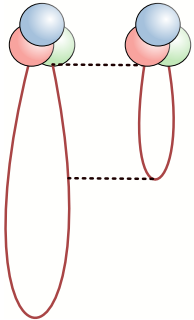
Filter Scores

- vRNA is looped



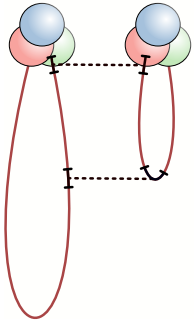
Filter Scores

- vRNA is looped
- binding at endings



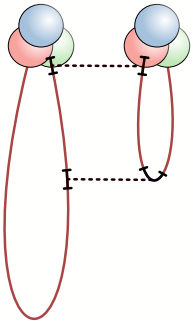
Filter Scores

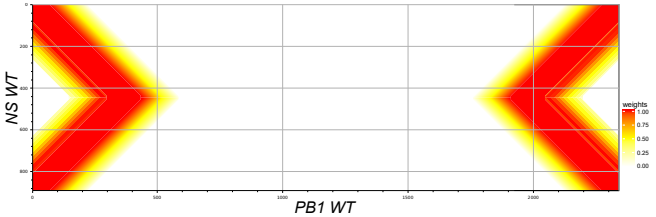
- vRNA is looped
- binding at endings
- some positional shift



Filter Scores

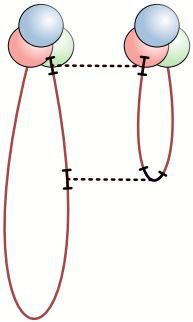
- vRNA is looped
- binding at endings
- some positional shift
- weight matrix

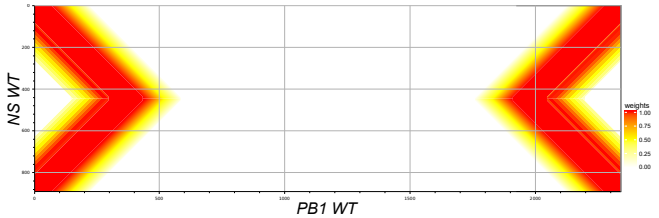


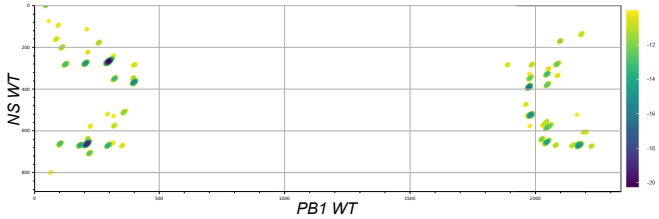


Filter Scores

- vRNA is looped
- binding at endings
- some positional shift
- weight matrix

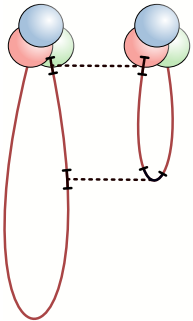


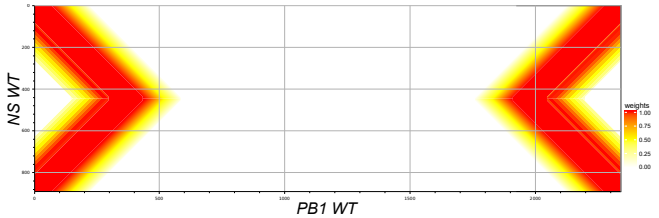


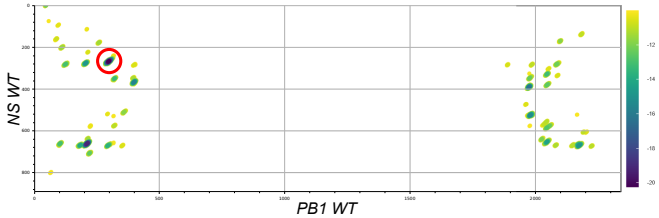


Filter Scores

- vRNA is looped
- binding at endings
- some positional shift
- weight matrix







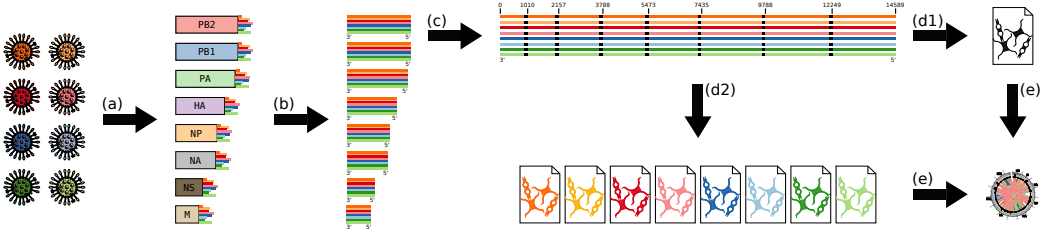
IAV Strains

Human	H1N1 WSN	(A/WSN/1933 TS61)
	H3N2 UTS	(A/Tokyo/Ut-Sk-1/2007)
Swine	H1N1 PR8	(A/Puerto Rico/8/34)
	H9N2 S15	(A/swine/Guangxi/S15/2005)
Avian	H5N1 UT6	(A/chicken/South Kalimantan/UT6028/2006)
	H6N6 165	(A/duck/Fujian/1651/2006)
Seal	H4N6 T12	(A/Caspian seal/Russia/T1/2012)
	H7N7 1SC	(A/seal/Mass/1-SC35M/1980)

Finding vRNA-vRNA Interactions

8 different strains

(a) split vRNA segments
(b) align each segment type
(c) concatenate alignments
(d1) multi LRIscan
(d2) single LRIscan
(e) visualize with circos



LRIscan Overview

(a) calculate alignment scores

- coverage
- complexity

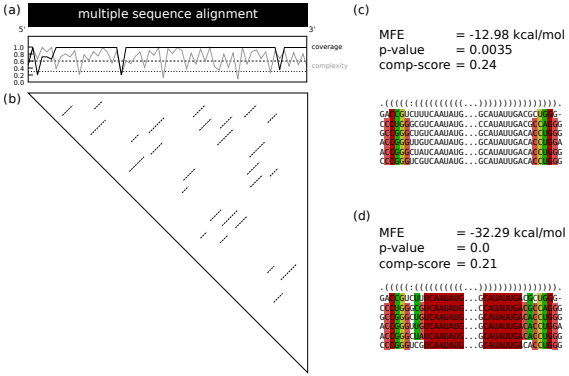
(b) RNAalifold seed finding via dotplot

- no gaps
- min length
- min distance

(c) seed filtering

- p-value
- minimum free energy (MFE)
- compensatory score

(d) seed extension



[Fricke and Marz, 2016]

Multi LRIscan

properties

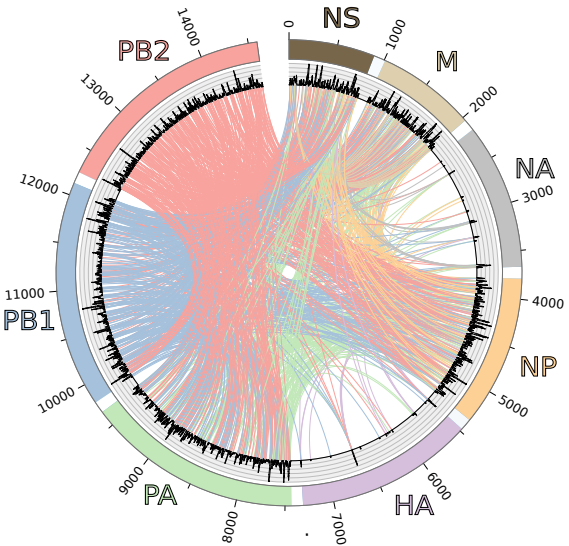
- MFE $\leq$ -10 kcal/mol
- link colors = segments

observations

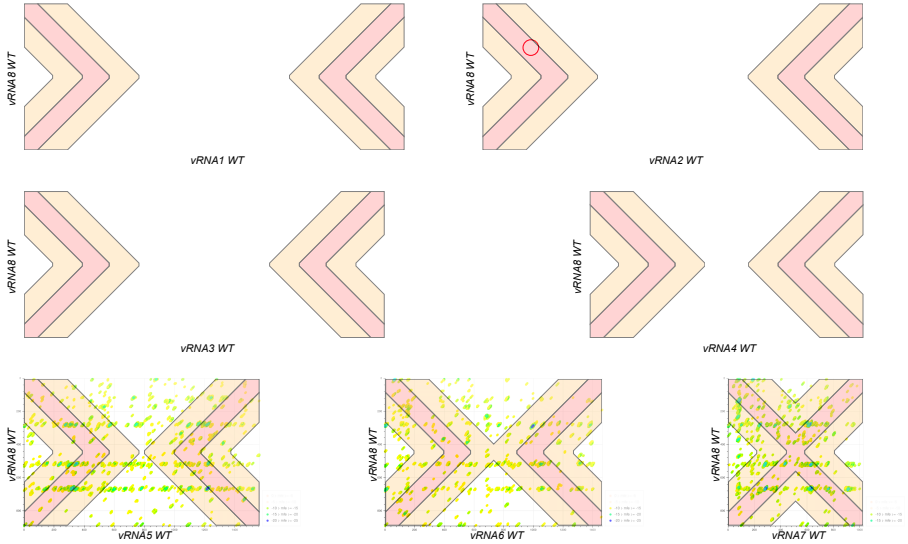
- vast # of links
- fewer links on HA + NA

interpretation

- constant evolutionary interactions
- HA + NA strain/host dependent
- supports reassortment



Hot Spots ?


vRNA8 WT
vRNA1 WT
vRNA8 WT
vRNA2 WT
vRNA8 WT
vRNA3 WT
vRNA8 WT
vRNA4 WT
vRNA8 WT
vRNA5 WT
vRNA8 WT
vRNA6 WT
vRNA8 WT
vRNA7 WT

Hot Spot

641-701
AUGUAGCGUUUCUGUUUUGGAGGGAGUGGAGGUCCCCCAU
491-551
UCUGUAGAAUGUCCUGGCAUGGAGGGAAUGGGAGAUAUUU
251-311
UCAGUGAUAUACCGAGGAGCAGGACUAGAGGCUAUGGCGA