

# SilentMutations (SIM): a tool for analyzing long-range RNA-RNA interactions in viral genomes and structured RNAs

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European Virus Bioinformatics Center, Jena

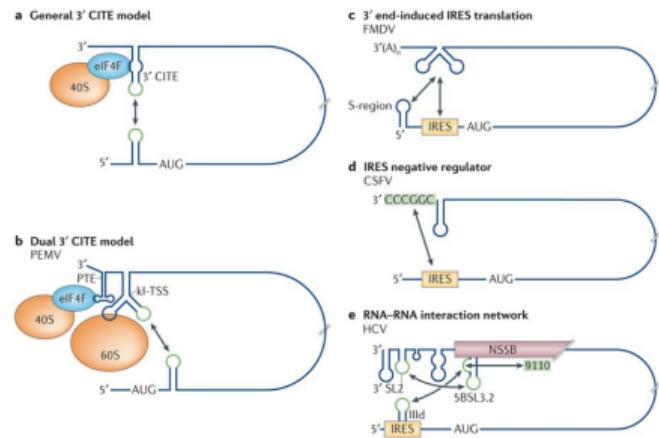
*daniel.desiro@uni-jena.de*

February 11, 2019

# Long-range RNA-RNA Interactions

## Translation Initiation

- 3'cap-independent translational enhancers
- internal ribosome entry sites



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[Nicholson and White, 2014, Functional long-range RNA-RNA interactions in positive-strand RNA viruses.]

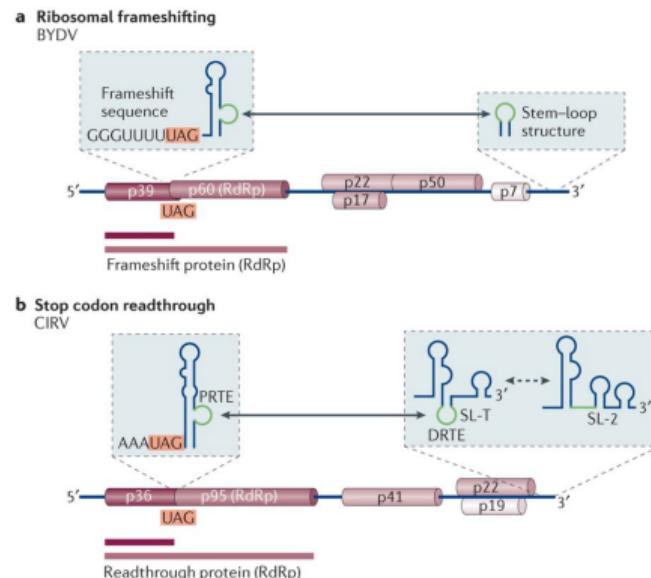
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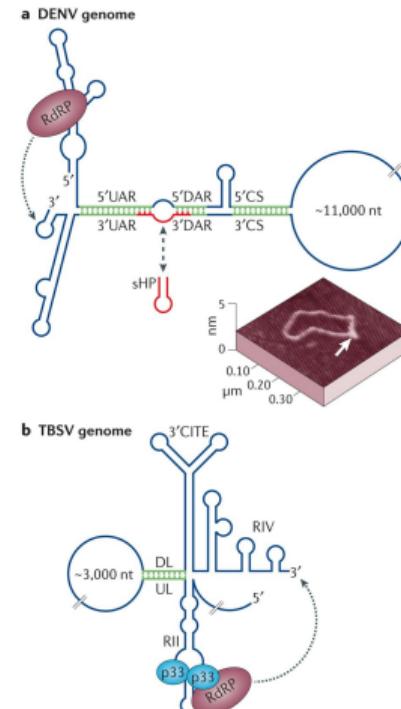
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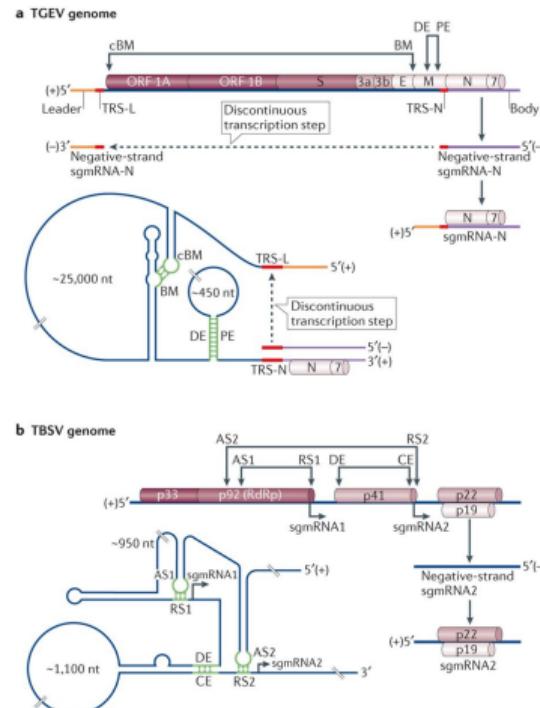
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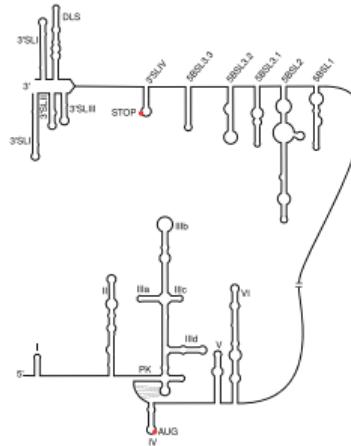
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Virus (genus)	RNA-RNA interaction	Viral process regulated	Refs
Many plant viruses in the Tombusviridae family and the Umbraviridae and Luteoviridae genera	3' CITE-5' UTR or 3' CITE-5' coding region	Translation initiation <sup>5,6</sup>	5,6
BYDV (Luteovirus)	3' CITE-5' UTR Frameshift site-3' UTR	Translation initiation Ribosomal frameshifting	21,25,30 47
CIRV (Tombusvirus)	3' CITE-5' UTR PRTE- DRTE	Translation initiation Stop codon readthrough	22,28 50
TBSV (Tombusvirus)	3' CITE-5' UTR UL-DL AS1-RS1; AS2-RS2; DE-CE	Translation initiation Genome replication sgmRNA transcription	26,27 52 81-84
FMDV (Aphthovirus)	IRES- 3' UTR S-region- 3' UTR	Translation initiation Possibly genome replication	32,33 33
CSFV (Pestivirus)	IRES- 3' terminus	Translation	34
HCV (Hepacivirus)	IRES-5BSL3.2 5BSL3.2- 3' UTR	Translation initiation Genome replication	35-37 38-42
DENV and WNV (Flavivirus)	5' UAR-3' UAR; 5' DAR-3' DAR; 5' CS-3' CS	Genome replication	53-61,65,66
TGEV (Coronavirus)	DE-PE; cBM-BM	sgmRNA-N transcription	77-79

# Conserved LRIs in Hepatitis C Viruses

## HCV Genome

- ssRNA+ ~10kb
- single polyprotein
- UTRs highly structured



[Fricke et al., 2015, Conserved RNA secondary structures and long-range interactions in hepatitis C viruses.]

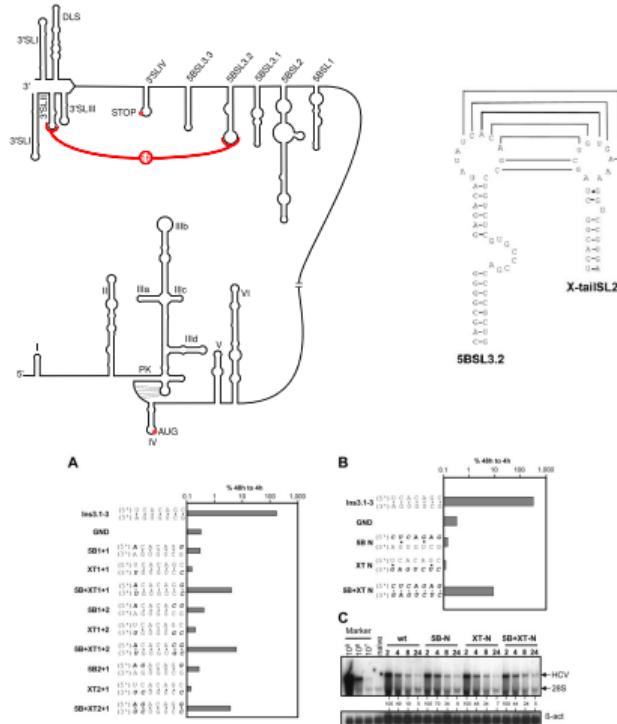
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  - LRI between 3'SLII and 5BSL3.2



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[Friebe et al., 2004, Kissing-loop interaction in the 3' end of the hepatitis C virus genome essential for RNA replication]

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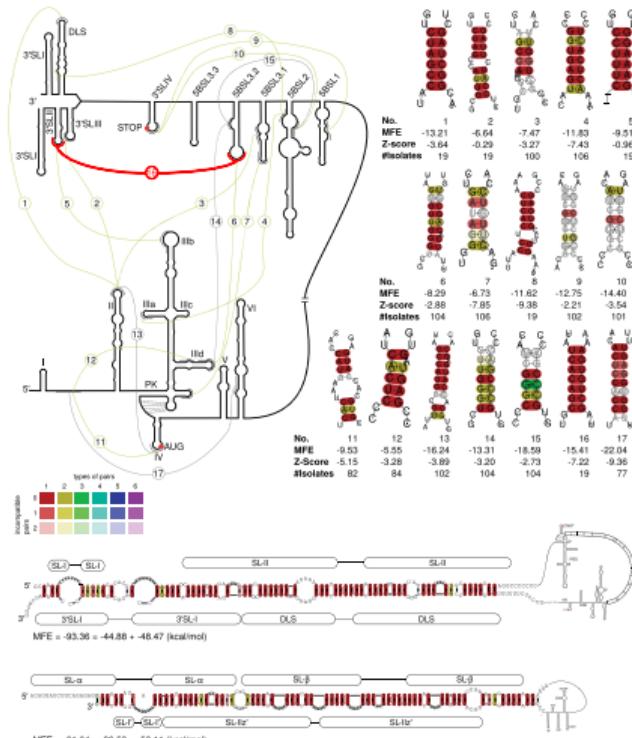
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## LRIsScan

- detected multiple LRIs
- verify several known
- identify several novel possible

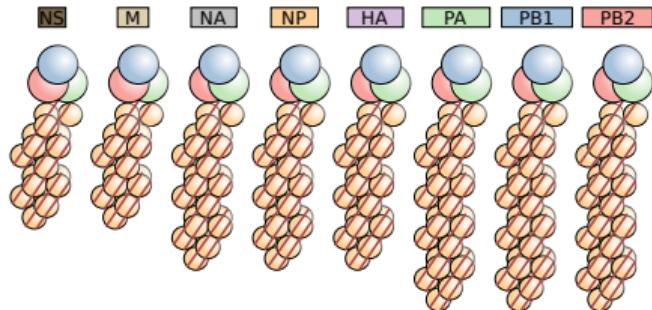


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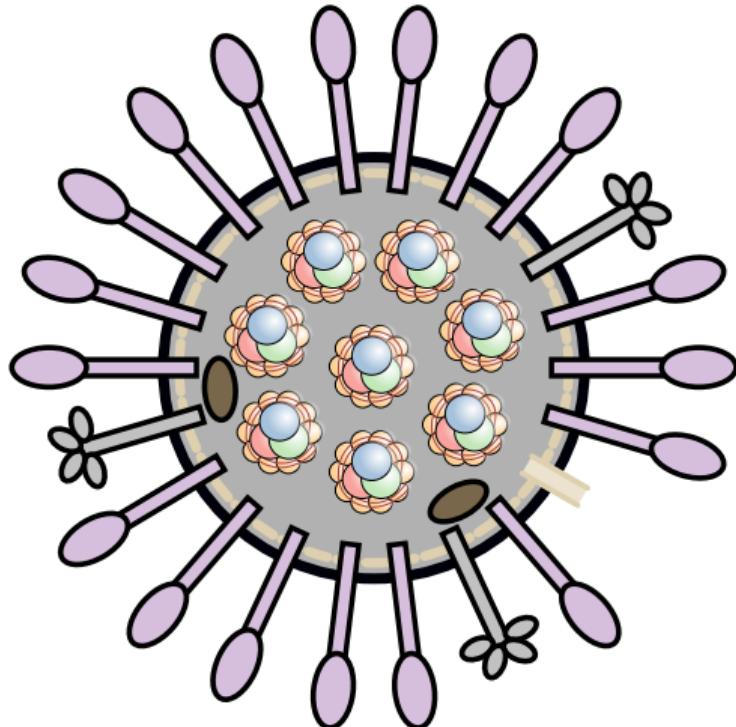
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# Influenza A Virus (IAV)

- polymerase basic proteins (PB2,PB1)
- polymerase acidic protein (PA)
- nucleoproteins (NP) + vRNA
- nuclear export proteins (NS)
- matrix protein (M) + lipid bilayer
- neuraminidase (NA) + hemagglutin (HA)

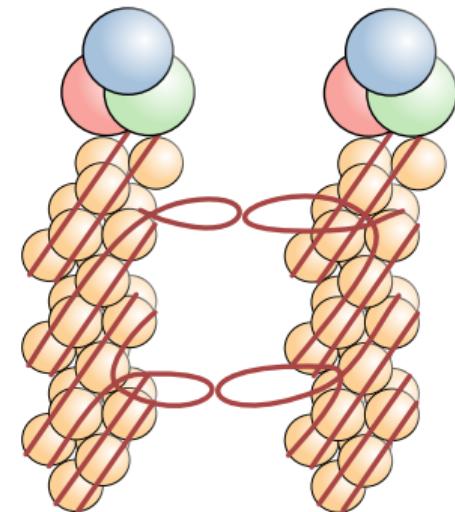
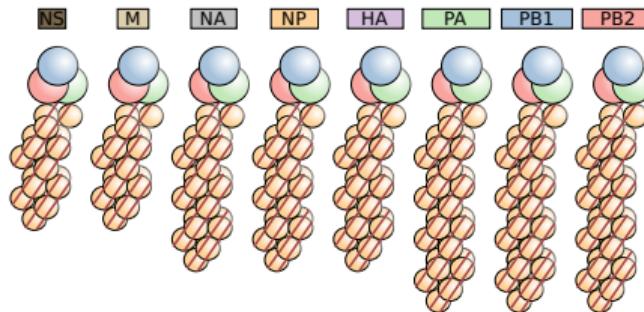


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# Verification Experiment

## Interaction

- avian H5N2
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[Gavazzi et al., 2013, A functional sequence-specific interaction between influenza a virus genomic RNA segments.]

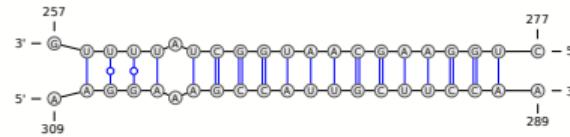
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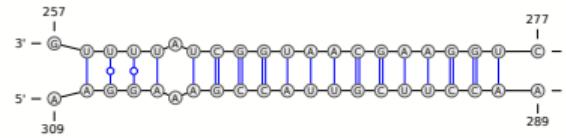
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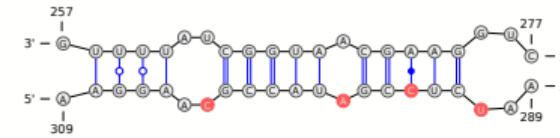
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-14.20 kcal/mol



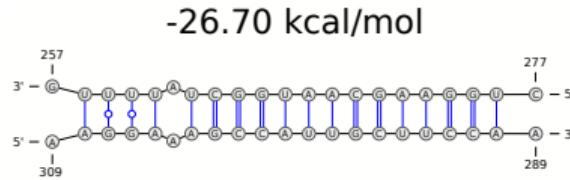
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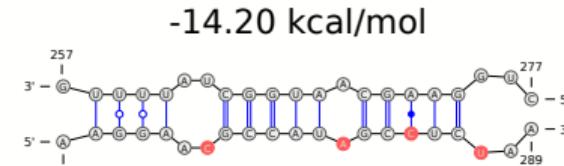
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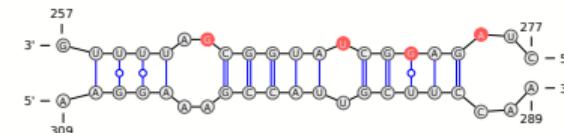
LRlscan



-26.70 kcal/mol



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-16.10 kcal/mol

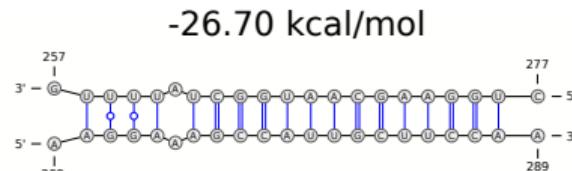
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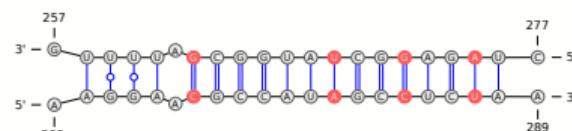
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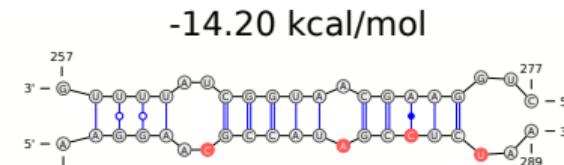
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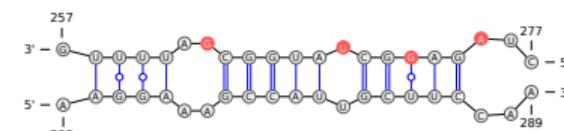
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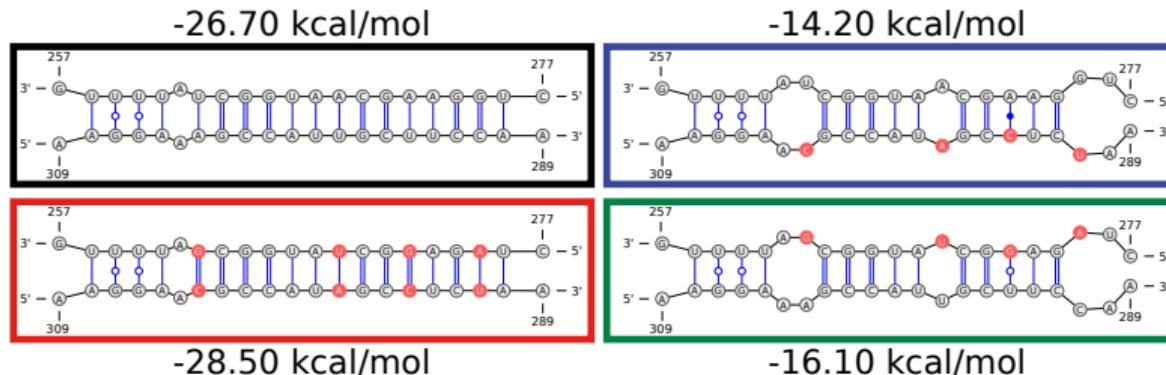
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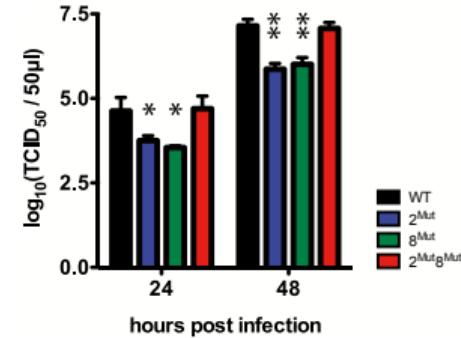
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Gavazzi et al.

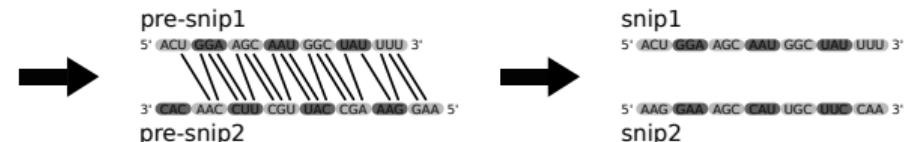


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# SIM Workflow

## (a) preprocessing

seq1:2:29-45  
5' ●●●●●●● CUA (AUA) CCG (AGG) AGC (AGG) ACU (GGG) ACC (AAU) GGC (UAU) UUU (GAC) GUU (UAC) ●●●●●●● 3'  
seq2:1:21-42  
5' ●●●●●●● CUA (GGA) UUC (UUC) AAG (GAA) AGC (GAA) UGC (UUC) CAA (GAA) ACA (AUC) UGU (UAC) ●●●●●●● 3'

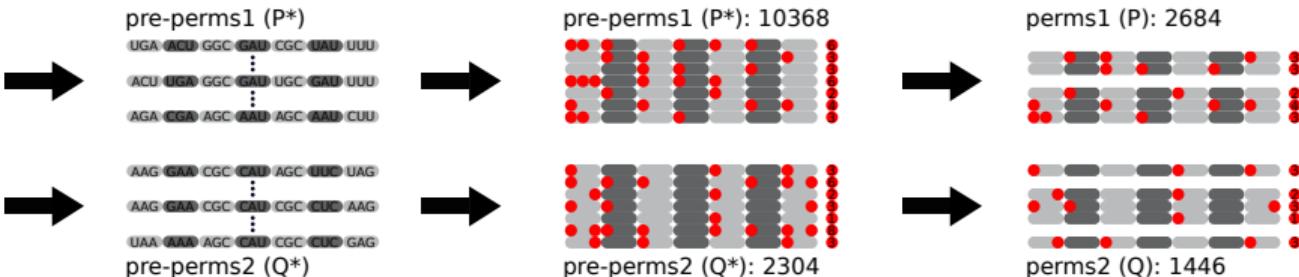


[Lorenz et al., 2011, ViennaRNA package 2.0.]

# SIM Workflow

## (b) permutation

codons1 (reverse complement)  
  
codons2 (reverse complement)



[Lorenz et al., 2011, ViennaRNA package 2.0.]

# SIM Workflow

## (c) attenuation

snip1  
5' ACU CGG AGC UAU GGC UAU UUU 3'  
  
perm<sub>2j</sub> ( $\in Q$ )  
perm<sub>1i</sub> ( $\in P$ )  
5' ACU CGG GGC GAU UGG (GAU) UUU 3'  
  
3' AAC CGG CGU UAC CGA UAG GAA 5'  
snip2

upper limit (I)

snip1

5' ACU CGG AGC UAU GGC UAU UUU 3'

3' AAC CGG CGU UAC CGA UAG GAA 5'

snip2

• prc

mut1 (U): 1029

&lt;img alt="Diagram of mutation U showing red dots at positions 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 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2491, 2493, 2495, 2497, 2499, 2501, 2503, 2505, 2507, 2509, 2511, 2513, 2515, 2517, 2519, 2521, 2523, 2525, 2527, 2529, 2531, 2533, 2535, 2537, 2539, 2541, 2543, 2545, 2547, 2549, 2551, 2553, 2555, 2557, 2559, 2561, 2563, 2565, 2567, 2569, 2571, 2573, 2575, 2577, 2579, 2581, 2583, 2585, 2587, 2589, 2591, 2593, 2595, 2597, 2599, 2601, 2603, 2605, 2607, 2609, 2611, 2613, 2615, 2617, 2619, 2621, 2623, 2625, 2627, 2629, 2631, 2633, 2635, 2637, 2639, 2641, 2643, 2645, 2647, 2649, 2651, 2653, 2655, 2657, 2659, 2661, 2663, 2665, 2667, 2669, 2671, 2673, 2675, 2677, 2679, 2681, 2683, 2685, 2687, 2689, 2691, 2693, 2695, 2697, 2699, 2701, 2703, 2705, 2707, 2709, 2711, 2713, 2715, 2717, 2719, 2721, 2723, 2725, 2727, 2729, 2731, 2733, 2735, 2737, 2739, 2741, 2743, 2745, 2747, 2749, 2751, 2753, 2755, 2757, 2759, 2761, 2763, 2765, 2767, 2769, 2771, 2773, 2775, 2777, 2779, 2781, 2783, 2785, 2787, 2789, 2791, 2793, 2795, 2797, 2799, 2801, 2803, 2805, 2807, 2809, 2811, 2813, 2815, 2817, 2819, 2821, 2823, 2825, 2827, 2829, 2831, 2833, 2835, 2837, 2839, 2841, 2843, 2845, 2847, 2849, 2851, 2853, 2855, 2857, 2859, 2861, 2863, 2865, 2867, 2869, 2871, 2873, 2875, 2877, 2879, 2881, 2883, 2885, 2887, 2889, 2891, 2893, 2895, 2897, 2899, 2901, 2903, 2905, 2907, 2909, 2911, 2913, 2915, 2917, 2919, 2921, 2923, 2925, 2927, 2929, 2931, 2933, 2935, 2937, 2939, 2941, 2943, 2945, 2947, 2949, 2951, 2953, 2955, 2957, 2959, 2961, 2963, 2965, 2967, 2969, 2971, 2973, 2975, 2977, 2979, 2981, 2983, 2985, 2987, 2989, 2991, 2993, 2995, 2997, 2999, 3001, 3003, 3005, 3007, 3009, 3011, 3013, 3015, 3017, 3019, 3021, 3023, 3025, 3027, 3029, 3031, 3033, 3035, 3037, 3039, 3041, 3043, 3045, 3047, 3049, 3051, 3053, 3055, 3057, 3059, 3061, 3063, 3065, 3067, 3069, 3071, 3073, 3075, 3077, 3079, 3081, 3083, 3085, 3087, 3089, 3091, 3093, 3095, 3097, 3099, 3101, 3103, 3105, 3107, 3109, 3111, 3113, 3115, 3117, 3119, 3121, 3123, 3125, 3127, 3129, 3131, 3133, 3135, 3137, 3139, 3141, 3143, 3145, 3147, 3149, 3151, 3153, 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3487, 3489, 3491, 3493, 3495, 3497, 3499, 3501, 3503, 3505, 3507, 3509, 3511, 3513, 3515, 3517, 3519, 3521, 3523, 3525, 3527, 3529, 3531, 3533, 3535, 3537, 3539, 3541, 3543, 3545, 3547, 3549, 3551, 3553, 3555, 3557, 3559, 3561, 3563, 3565, 3567, 3569, 3571, 3573, 3575, 3577, 3579, 3581, 3583, 3585, 3587, 3589, 3591, 3593, 3595, 3597, 3599, 3601, 3603, 3605, 3607, 3609, 3611, 3613, 3615, 3617, 3619, 3621, 3623, 3625, 3627, 3629, 3631, 3633, 3635, 3637, 3639, 3641, 3643, 3645, 3647, 3649, 3651, 3653, 3655, 3657, 3659, 3661, 3663, 3665, 3667, 3669, 3671, 3673, 3675, 3677, 3679, 3681, 3683, 3685, 3687, 3689, 3691, 3693, 3695, 3697, 3699, 3701, 3703, 3705, 3707, 3709, 3711, 3713, 3715, 3717, 3719, 3721, 3723, 3725, 3727, 3729, 3731, 3733, 3735, 3737, 3739, 3741, 3743, 3745, 3747, 3749, 3751, 3753, 3755, 3757, 3759, 3761, 3763, 3765, 3767, 3769, 3771, 3773, 3775, 3777, 3779, 3781, 3783, 3785, 3787, 3789, 3791, 3793, 3795, 3797, 3799, 3801, 3803, 3805, 3807, 3809, 3811, 3813, 3815, 3817, 3819, 3821, 3823, 3825, 3827, 3829, 3831, 3833, 3835, 3837, 3839, 3841, 3843, 3845, 3847, 3849, 3851, 3853, 3855, 3857, 3859, 3861, 3863, 3865, 3867, 3869, 3871, 3873, 3875, 3877, 3879, 3881, 3883, 3885, 3887, 3889, 3891, 3893, 3895, 3897, 3899, 3901, 3903, 3905, 3907, 3909, 3911, 3913, 3915, 3917, 3919, 3921, 3923, 3925, 3927, 3929, 3931, 3933, 3935, 3937, 3939, 3941, 3943, 3945, 3947, 3949, 3951, 3953, 3955, 3957, 3959, 3961, 3963, 3965, 3967, 3969, 3971, 3973, 3975, 3977, 3979, 3981, 3983, 3985, 3987, 3989, 3991, 3993, 3995, 3997, 3999, 4001, 4003, 4005, 4007, 4009, 4011, 4013, 4015, 4017, 4019, 4021, 4023, 4025, 4027, 4029, 4031, 4033, 4035, 4037, 4039, 4041, 4043, 4045, 4047, 4049, 4051, 4053, 4055, 4057, 4059, 4061, 4063, 4065, 4067, 4069, 4071, 4073, 4075, 4077, 4079, 4081, 4083, 4085, 4087, 4089, 4091, 4093, 4095, 4097, 4099, 4101, 4103,

# SIM Workflow

## (d) recovery

snip1  
5' (ACU GGA) AGC AAU GCC UAU UUU 3'  
||| / | / | / | / | / | / |  
3' AAC UGU CGU UGU CGA AAU GAA 5'  
snip2

• ldv



mut1<sub>i</sub> ( $\in$  U)  
5' (ACU GGA) GGC AAU UCC GAA UUU 3'  
||| / | / | / | / | / |  
3' GAA UGU CGC UAU CGC AAU GAA 5'  
mut2<sub>j</sub> ( $\in$  V)



snip1  
5' (ACU GGA) AGC AAU GCC UAU UUU 3'  
||| / | / | / | / | / |  
3' AAC UGU CGU UGU CGA AAU GAA 5'  
snip2

• udv



(mut1,mut2)<sub>i</sub> ( $\in$  M)  
[red dots] [grey bar]  
⋮  
[red dots] [grey bar]

[Lorenz et al., 2011, ViennaRNA package 2.0.]

# SIM Workflow

(e) sampling

$$\min \left( \begin{array}{c} \text{l-mrg} \geq \text{snip1} \\ \text{mut2}_i (\in M) \\ \text{l-mrg} \geq \text{u-mrg} \\ + \\ \text{l-mrg} \geq \text{mut1}_i (\in M) \\ \text{snip2} \\ \text{l-mrg} \geq \text{u-mrg} \end{array} \right)$$

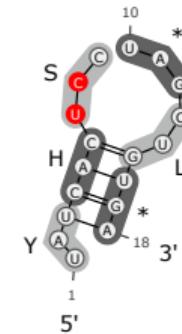
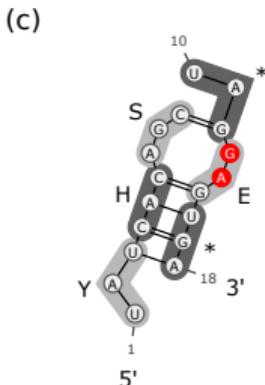
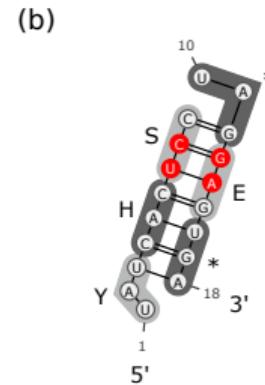
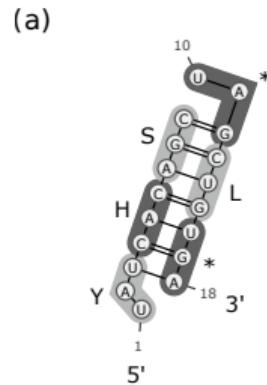
The diagram shows two RNA sequences: snip1 and snip2. snip1 is a 5' to 3' sequence with mutations at positions 1, 3, 5, 7, 9, and 11. mut2<sub>i</sub> (in M) is a 3' to 5' sequence with mutations at positions 1, 3, 5, 7, 9, and 11. mut1<sub>i</sub> (in M) is a 5' to 3' sequence with mutations at positions 1, 3, 5, 7, 9, and 11. The l-mrg and u-mrg values are determined by comparing these sequences.

$$\text{l-mrg} = (mfe(\text{snip1}, \text{mut2}_i) + mfe(\text{mut1}_i, \text{snip2})) \cdot 0.5 \cdot (1 + -\text{mrg})$$

$$\text{u-mrg} = (mfe(\text{snip1}, \text{mut2}_i) + mfe(\text{mut1}_i, \text{snip2})) \cdot 0.5 \cdot (1 - -\text{mrg})$$

[Lorenz et al., 2011, ViennaRNA package 2.0.]

# HCV Result



$$mfe(5BSL3.2_{WT}, SLII_{WT}) = -9.70 \text{ kcal/mol}$$

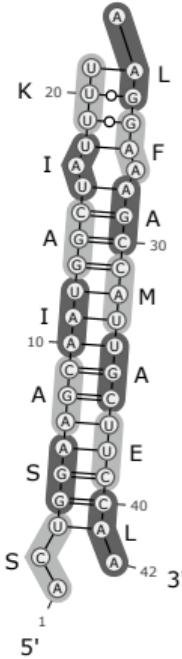
$$mfe(5BSL3.2_{mut}, SLII_{mut}) = -9.90 \text{ kcal/mol}$$

$$mfe(5BSL3.2_{WT}, SLII_{mut}) = -2.40 \text{ kcal/mol}$$

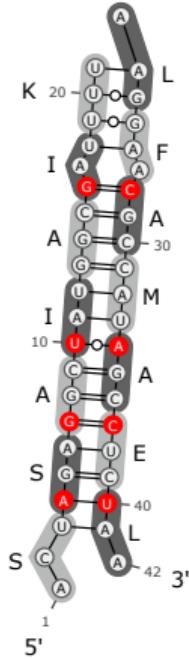
$$mfe(5BSL3.2_{mut}, SLII_{WT}) = -2.10 \text{ kcal/mol}$$

[Darty et al., 2009, VARNA: Interactive drawing and editing of the RNA secondary structure..]

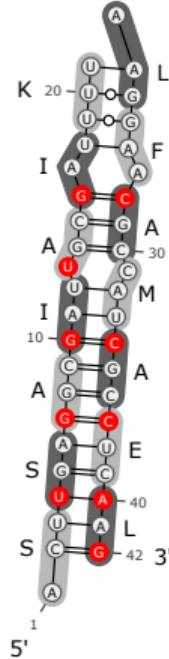
# IAV Result



$$\text{mfe}(\text{NS}_{\text{WT}}, \text{PB1}_{\text{WT}}) = -24.60 \text{ kcal/mol}$$



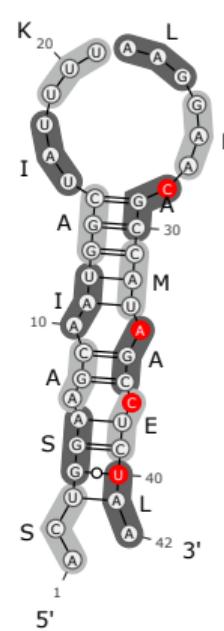
$$\text{mfe}(\text{NS}_{\text{mut}}, \text{PB1}_{\text{WT}}) = -26.40 \text{ kcal/mol}$$



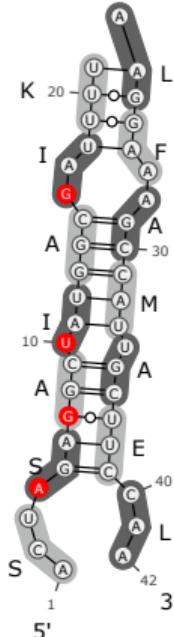
$$\text{mfe}(\text{NS}_{\text{mut}}, \text{PB1}_{\text{mut}}) = -23.40 \text{ kcal/mol}$$

[Darty et al., 2009, VARNA: Interactive drawing and editing of the RNA secondary structure..]

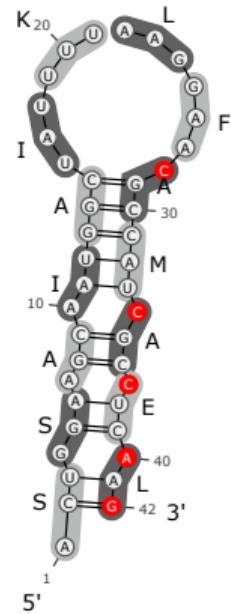
# IAV Result



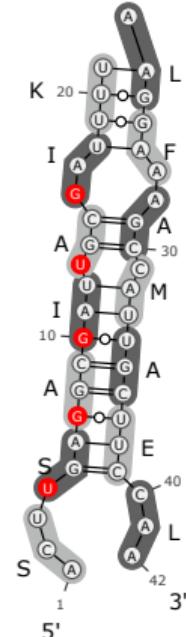
$$\text{mfe}(\text{NS}_{\text{mut}}, \text{PB1}_{\text{WT}}) = -11.80 \text{ kcal/mol}$$



$$\text{mfe}(\text{NS}_{\text{WT}}, \text{PB1}_{\text{mut}}) = -14.00 \text{ kcal/mol}$$



$$\text{mfe}(\text{NS}_{\text{WT}}, \text{PB1}_{\text{mut}}) = -10.10 \text{ kcal/mol}$$



$$\text{mfe}(\text{NS}_{\text{mut}}, \text{PB1}_{\text{WT}}) = -11.30 \text{ kcal/mol}$$

[Darty et al., 2009, VARNA: Interactive drawing and editing of the RNA secondary structure..]

# Discussion

## Dependencies

- Python (v3.6.5)
- ViennaRNA Package (v2.4)
- VARNA (v3.93) (optional)

[Fricke and Marz, 2016, Prediction of conserved long-range RNA-RNA interactions in full viral genomes.]

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## Run Time

- worst case:  $O(c^{\frac{k}{3} + \frac{l}{3}})$
- highly dependent on filter steps
- Example:  $10368 \cdot 2304$  to  $1029 \cdot 36$

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## Summary

- 4 way verification experiment
- ssRNA+ and ssRNA-
- coding and non-coding

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## Summary

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- ssRNA+ and ssRNA-
- coding and non-coding

## Application

- predict LRIs (LRlscan)
- simulate mutations (SIM)
- create mutants (Lab)

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- Manja Marz

