

Supplementary information

Crystal Structure of a Cap-Independent Translation Enhancer RNA

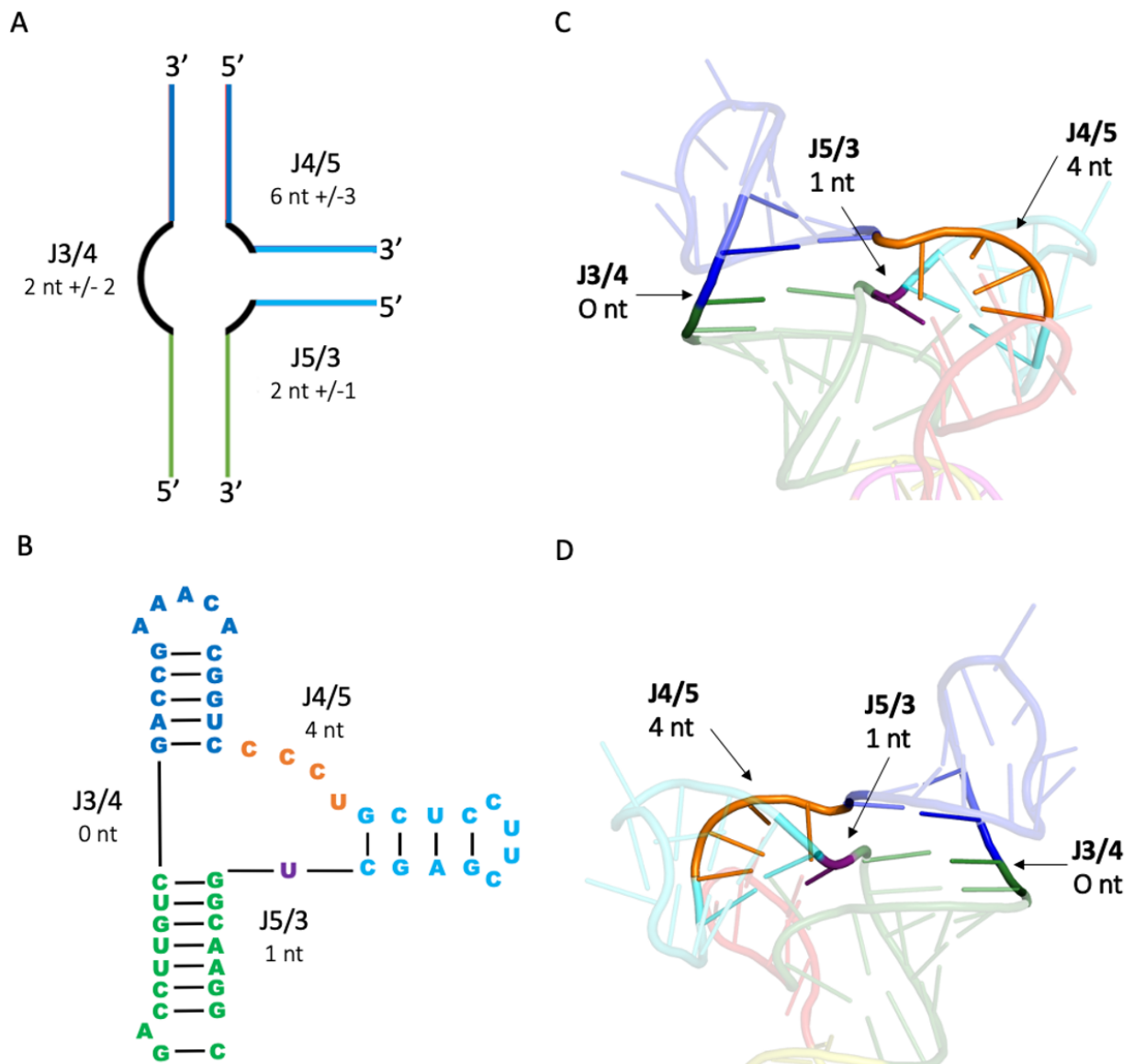
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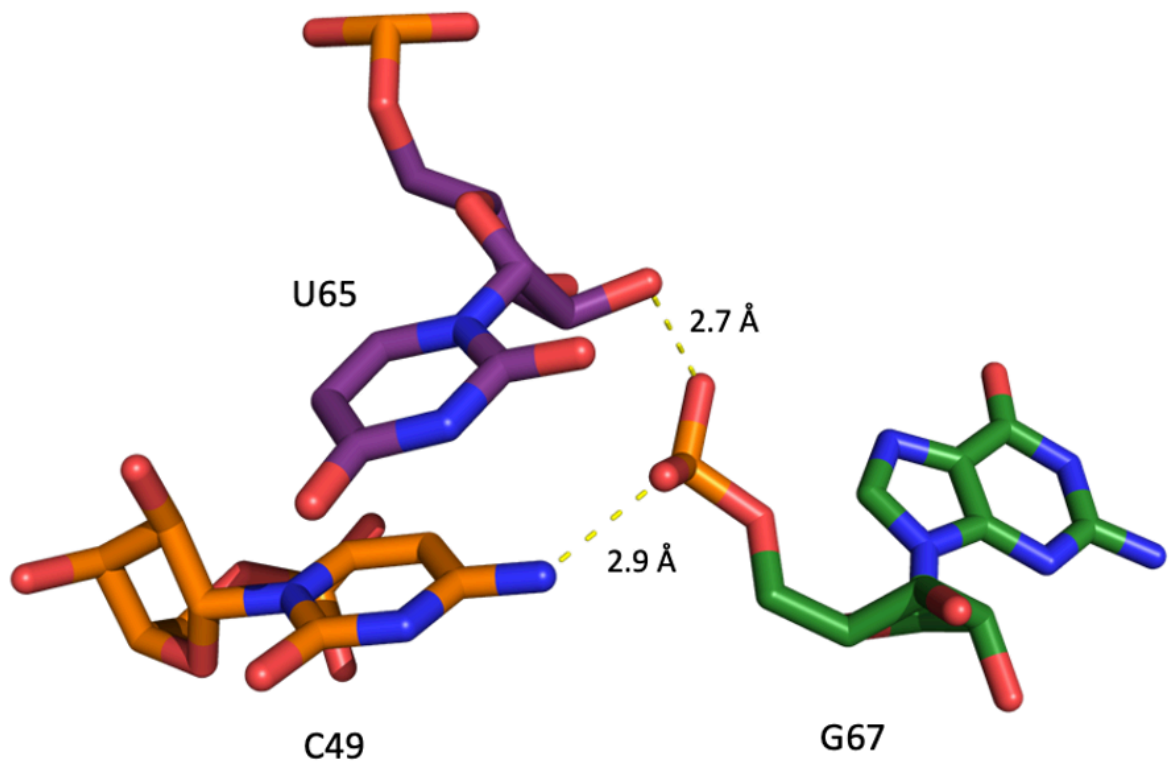
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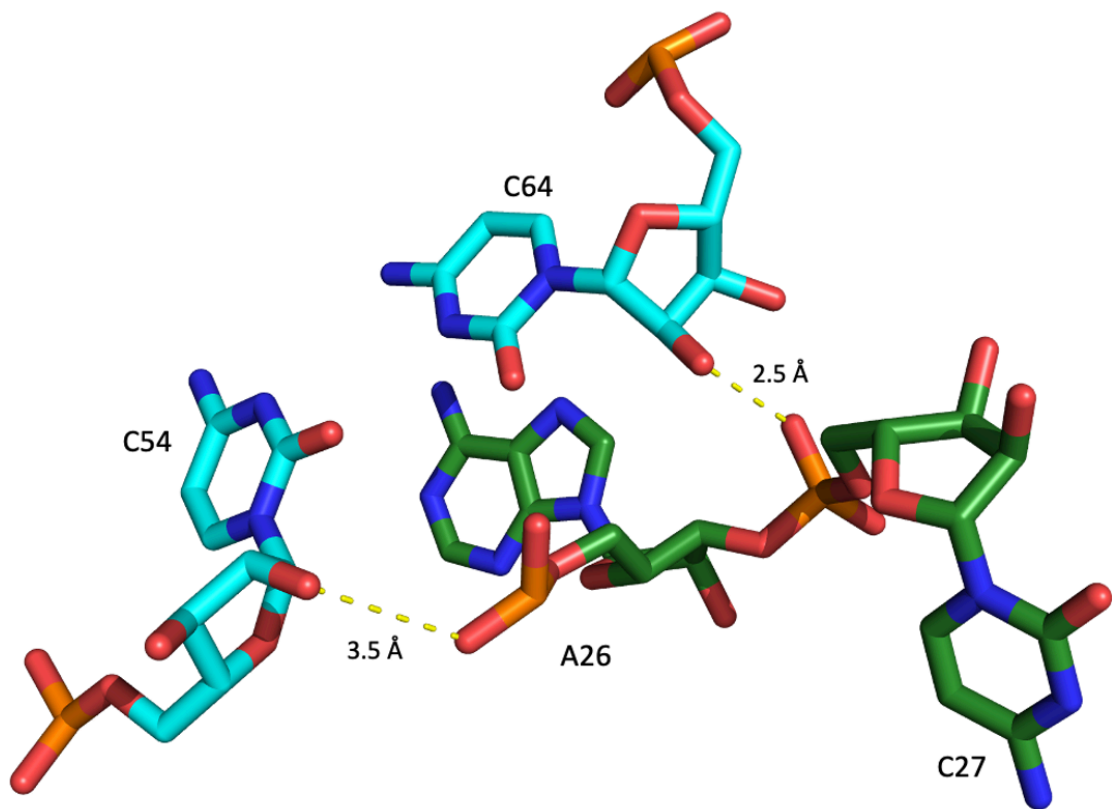
† Joint Authors



Supplementary Figure 1. (A) Schematic of a generalized A-type three-way junction illustrating the arrangement of P3, P4 and P5 and the observed lengths of joining regions (J) found in the canonical A-type junction (1). (B) Crystal-structure-derived secondary structure of PEMV2 focused on the A-type three-way junction. (C) Three-dimensional illustration of the PEMV2's three-way junction indicating the number of nucleotides (nt) in each junction between paired regions. (D) Same image as shown in C but rotated 180 degrees.

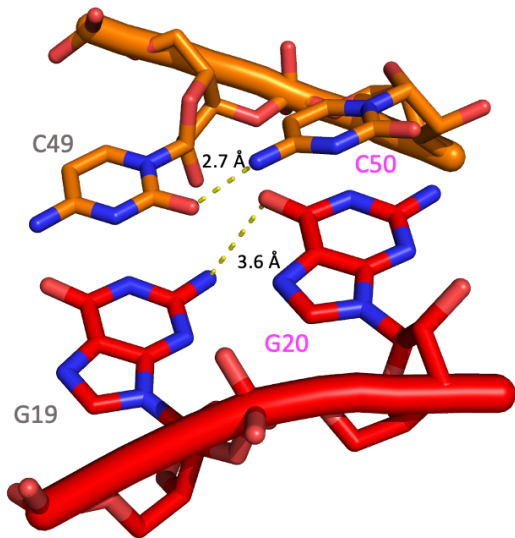


Supplementary Figure 2. Three-way junction/joining region interactions C49:N4 (J4/5) and G67:OP1 (P3), U65:2'OH (J5/3) and G67:OP2 (P3).

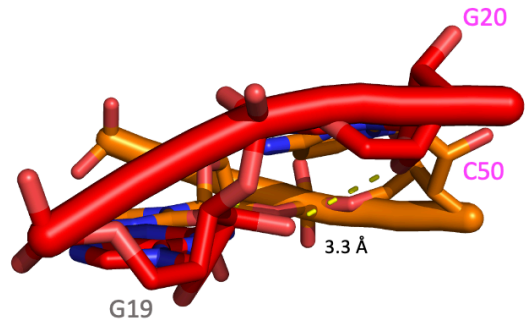


Supplementary Figure 3. Interactions between joining regions. A26:OP2 (P3) and C54:2'OH (P5), C27:OP1 (P3) and C64:2'OH (P5).

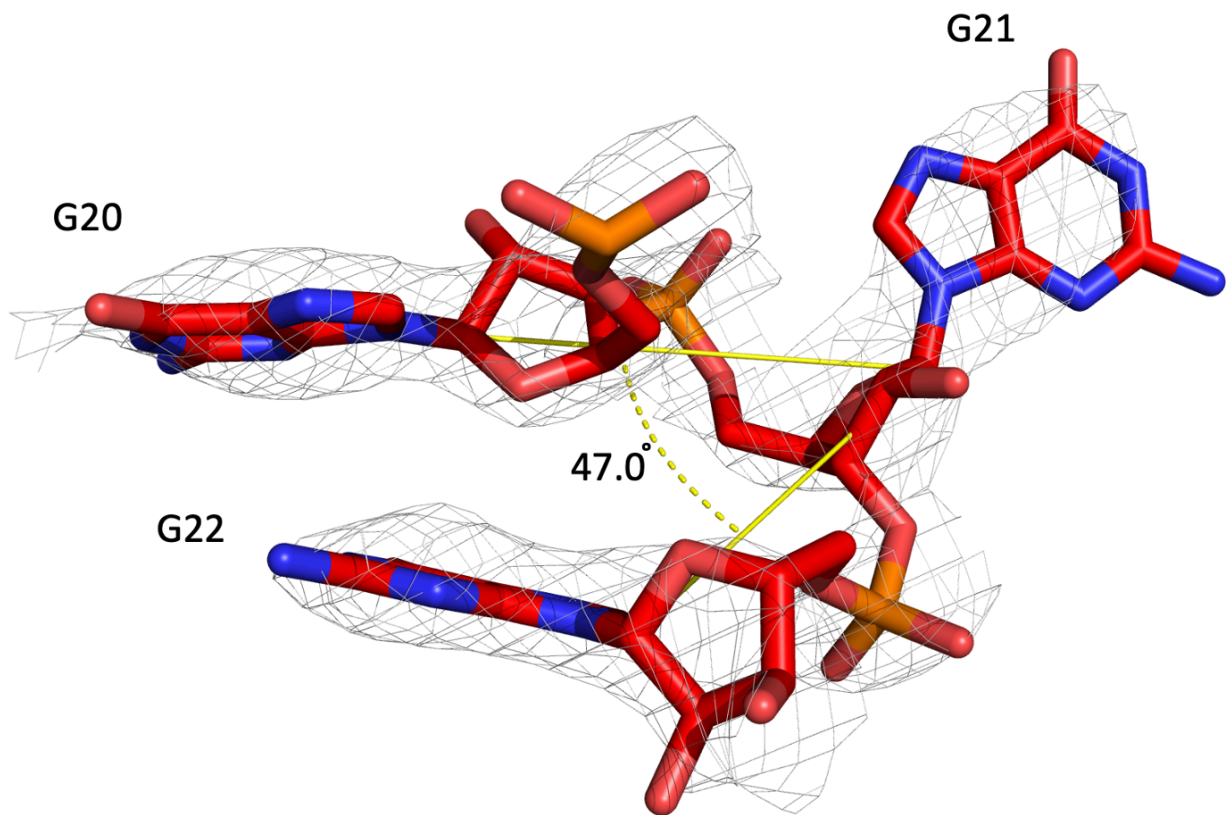
A



B

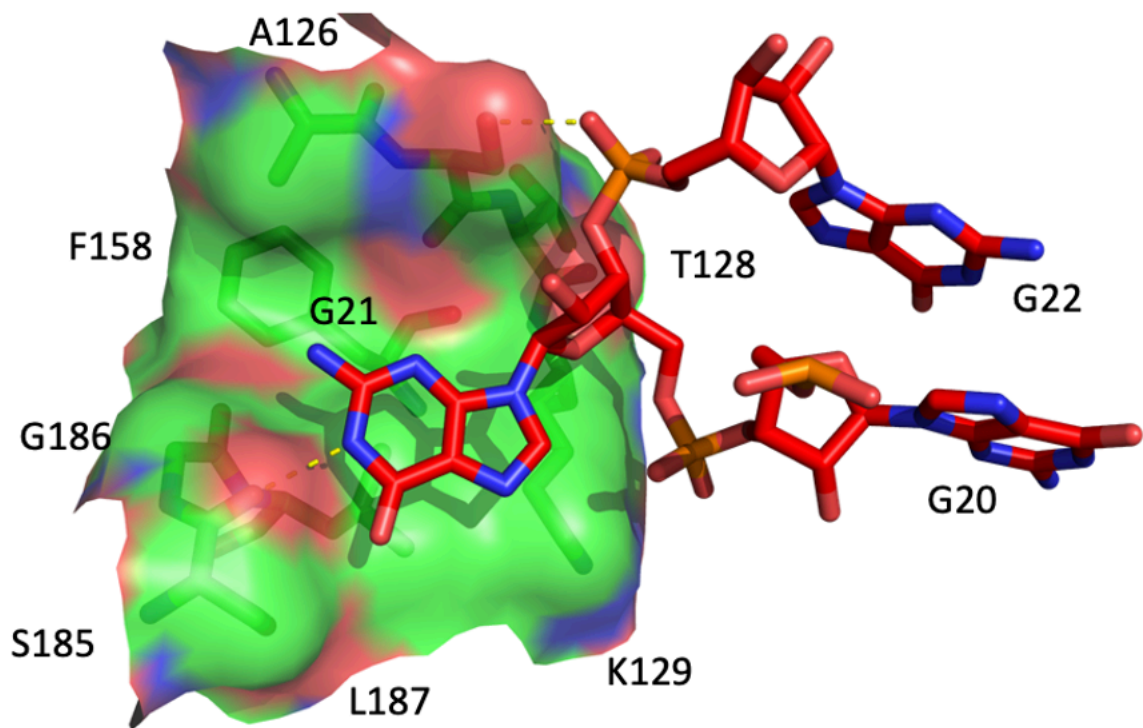


Supplementary Figure 4. (A) Parallel cis WCF pairing of G19-C49 (layer 2) and G20-C50 (layer 1) with depicted cross-layer interactions. G19:N2 (layer 2) interacts with G20:O6 (layer 1); G19:2'OH (layer 2) interacts with G20:O4' (layer 1); C49:O2 (layer 2) interacts with C50:N4 (layer 1). Residue denotation color analogous to its layer. (B) The image shown in A but rotated 45 degrees.

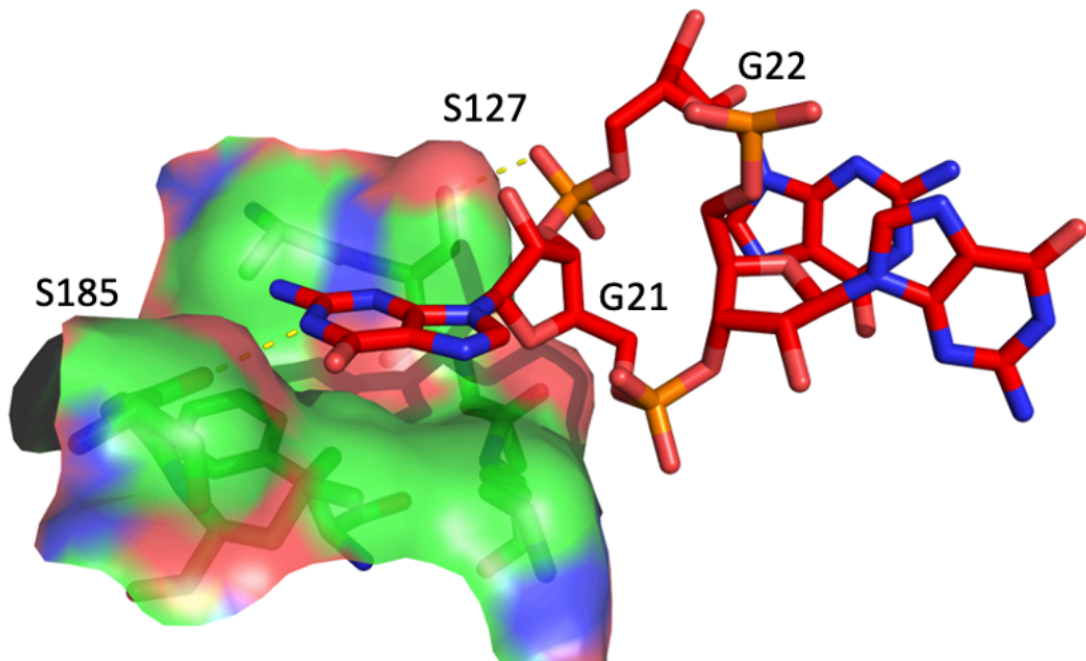


Supplementary Figure 5. Abrupt turn in the ribose phosphate backbone at G20, G2 and G22 with electron density map, contoured to 2.0 sigma. Yellow dashed arc indicates the angle formed by the intersection of lines (yellow) running through the ribose C1' atoms of G20, G21 and G22.

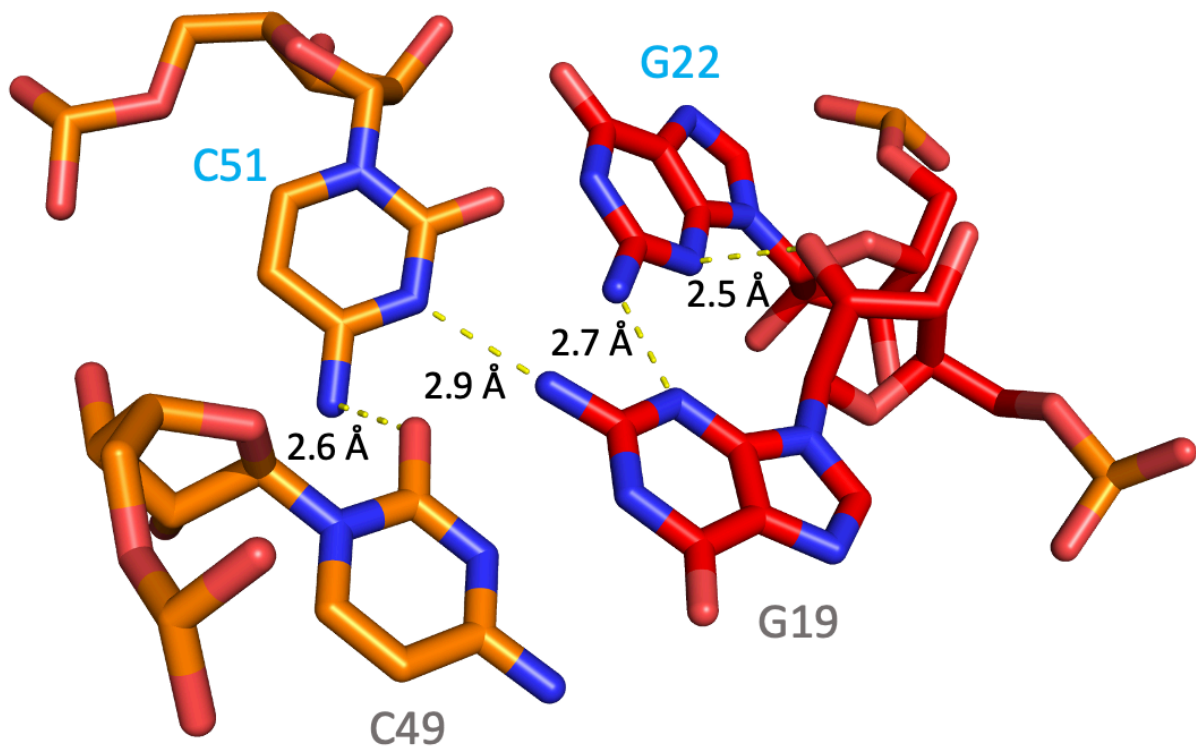
A



B

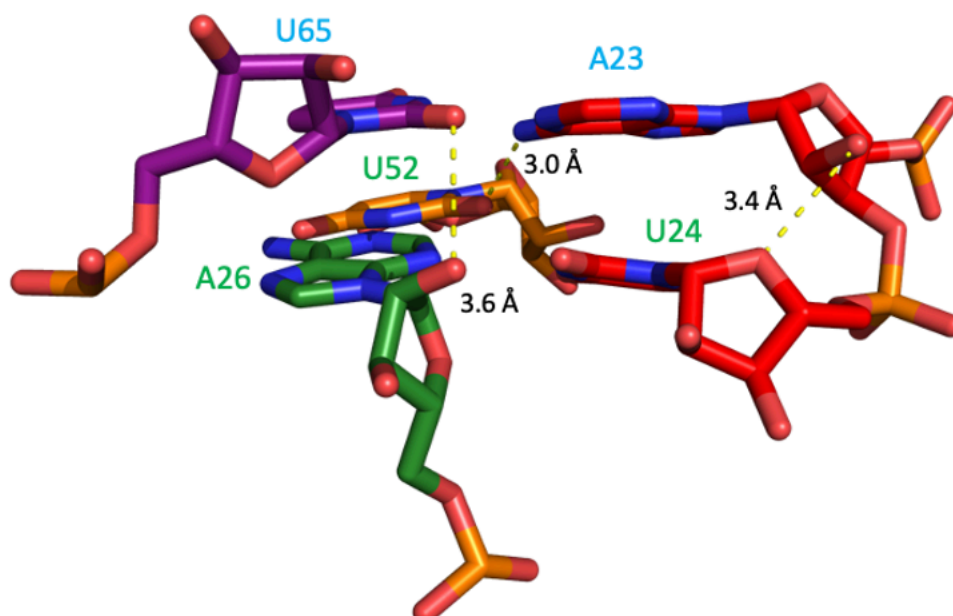


Supplementary Figure 6. (A) G21 and Fab heavy chain pocket (A126, S127, T128, K129, F158, S185, G186, L187). (B) The image shown in A but rotated 90 degrees.

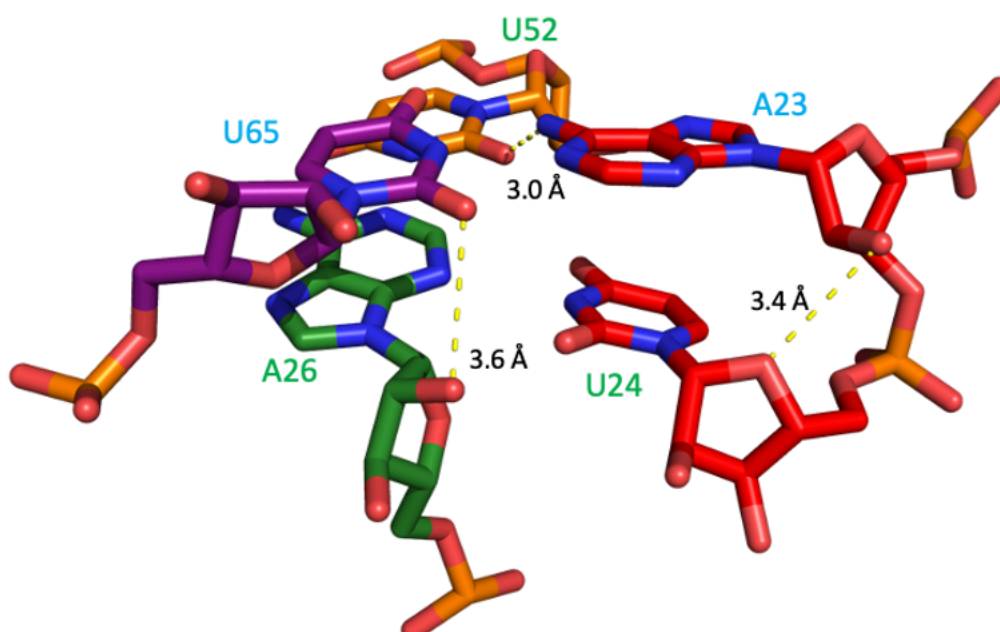


Supplementary Figure 7. Cross-layer interactions between layer 2 and layer 3. (A) G19:N3 and 2'OH (layer 2) reside within hydrogen bonding distance of G22:N2 and N3 (layer 3), respectively. G19:N2 interacts with C51:N3 (layer 3). C49:O2 (layer 2) reside within hydrogen bonding distance of C51:N4. Residue denotation color analogous to its layer.

A

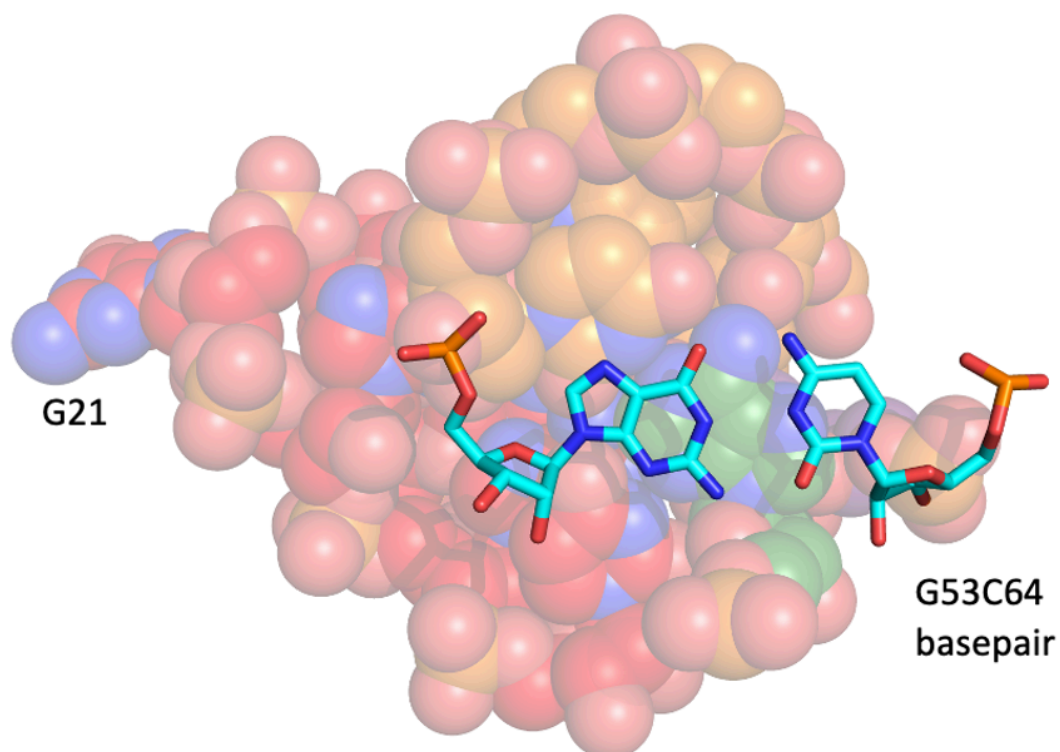


B

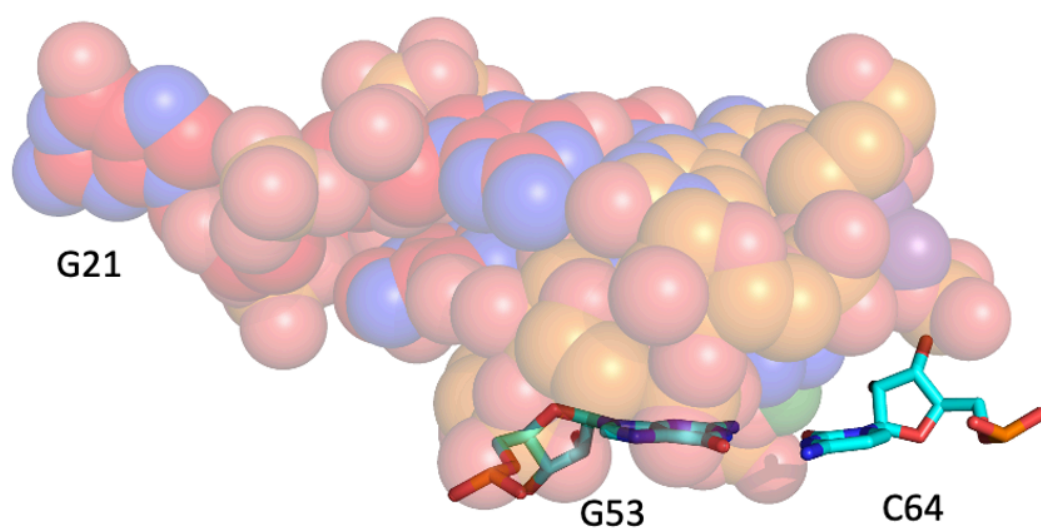


Supplementary Figure 8. Cross-layer interactions between layer 3 and 4. (A) U24:O4' and U52:O2 (both in layer 4) reside within hydrogen bonding distance of A23:2'OH and N6 (layer 3), respectively. U65:O2 (layer 3) interacts with A26:2'OH (layer 4). Residue denotation color analogous to its layer. (B) Same as A but rotated 30 degrees.

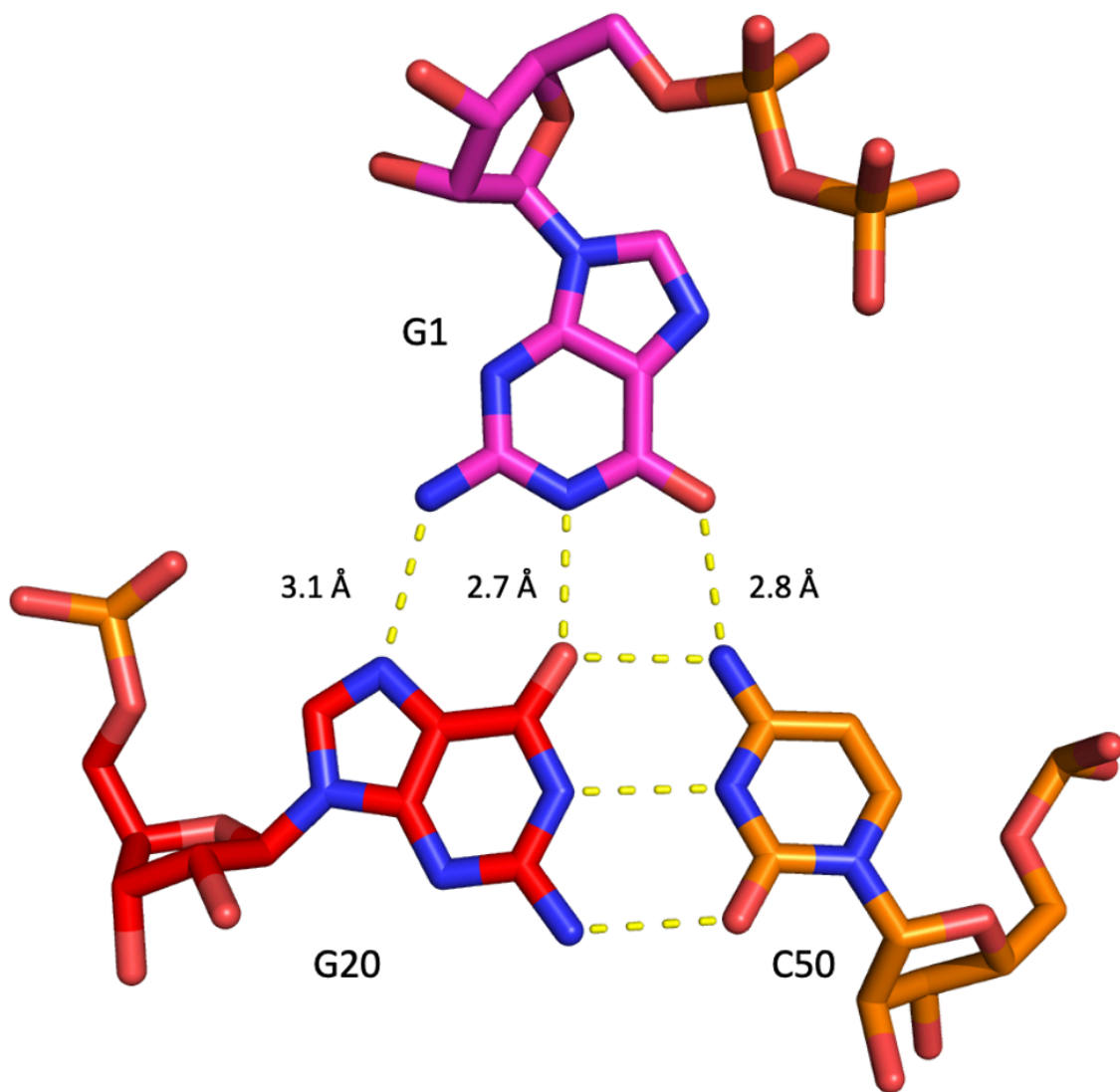
A



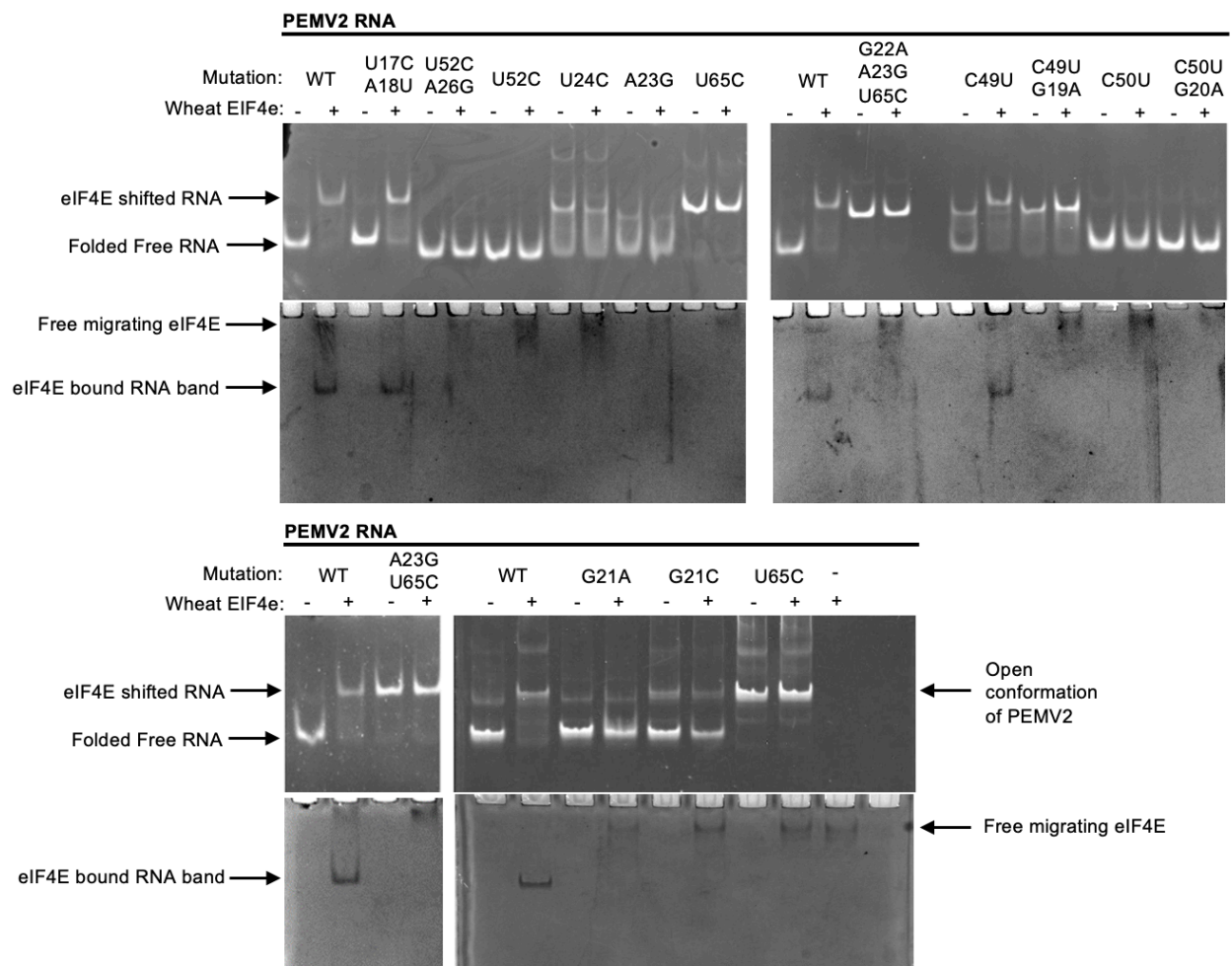
B



Supplementary Figure 9. (A) The G53-C64 basepair at the base of P5 forms a platform for the scaffold stack. (B) The image shown in A rotated 90 degrees.



Supplementary Figure 10. The G20-C50-G1 triple base crystal contact.



Supplementary Figure 11. Analysis of eIF4E complex formation with PEMV2 constructs by EMSA. Gel and running buffer contained 10 mM MgCl₂. RNA and protein were visualized using ethidium bromide and Coomassie staining, respectively. Mutations present in each tested construct are indicated above their respective lanes. Lanes labeled with a “-” contain RNA alone, while lanes labeled with a “+” contain 1.5-fold excess of recombinantly expressed wheat eIF4E in addition to the refolded RNA.

Supplementary Table 1. DNA Sequences of Oligonucleotides.

Construct name	Sequence
PEMV2_WT _crystallization	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGGGGATGACC TTGTCGACCGGTTATCGGTCCCCTGCTCCTTCGAGCTGGCAAGGCGCTCA CAGGTTCTACC
PEMV2_BL36 _crystallization	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGGGGATGACC TTGTCGACCGAAACACGGTCCCCTGCTCCTTCGAGCTGGCAAGGCGCTCA CAGGTTCTACC
EMV2_WT _EMSA	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGGGGATGACC TTGTCGACCGGTTATCGGTCCCCTGCTCCTTCGAGCTGGCAAGGCGCTCA CAGGTTCTAC
PEMV2_U17C_ A18U	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGACTGGGGATGACC TTGTCGACCGGTTATCGGTCCCCTGCTCCTTCGAGCTGGCAAGGCGCTCA CAGGTTCTAC
PEMV2_U52C_ A26G	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGGGGATGGCC TTGTCGACCGGTTATCGGTCCCCCGCTCCTTCGAGCTGGCAAGGCGCTCA CAGGTTCTAC
PEMV2_U52C	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGGGGATGACC TTGTCGACCGGTTATCGGTCCCCCGCTCCTTCGAGCTGGCAAGGCGCTCA CAGGTTCTAC
PEMV2_U24C	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGGGGACGAC CTTGTCGACCGGTTATCGGTCCCCTGCTCCTTCGAGCTGGCAAGGCGCTC ACAGGTTCTAC
PEMV2_A23G	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGGGGGTGACC TTGTCGACCGGTTATCGGTCCCCTGCTCCTTCGAGCTGGCAAGGCGCTCA CAGGTTCTAC
PEMV2_U65C	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGGGGATGACC TTGTCGACCGGTTATCGGTCCCCTGCTCCTTCGAGCCGGAAGGCGCTCA CAGGTTCTAC
PEMV2_G22A_ A23G_U65C	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGGGAGTGACC TTGTCGACCGGTTATCGGTCCCCTGCTCCTTCGAGCCGGAAGGCGCTCA CAGGTTCTAC
PEMV2_A23G_ U65C	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGGGGGTGACC TTGTCGACCGGTTATCGGTCCCCTGCTCCTTCGAGCCGGAAGGCGCTCA CAGGTTCTAC
PEMV2_C49U	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGGGGATGACC TTGTCGACCGGTTATCGGTCTCCTGCTCCTTCGAGCTGGCAAGGCGCTCA CAGGTTCTAC
PEMV2_C49U_ G19A	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAAGGGATGACC TTGTCGACCGGTTATCGGTCTCCTGCTCCTTCGAGCTGGCAAGGCGCTCA CAGGTTCTAC
PEMV2_C50U	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGGGGATGACC TTGTCGACCGGTTATCGGTCTCTGCTCCTTCGAGCTGGCAAGGCGCTCA CAGGTTCTAC
PEMV2_C50U_ G20A	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGAGGATGACC TTGTCGACCGGTTATCGGTCTCTGCTCCTTCGAGCTGGCAAGGCGCTCA CAGGTTCTAC
PEMV2_G21A	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGGAGATGACC TTGTCGACCGGTTATCGGTCCCCTGCTCCTTCGAGCTGGCAAGGCGCTCA CAGGTTCTAC
PEMV2_G21C	5'GCGTAATACGACTCACTATAGGTAGAACACGTGGGATAGGCGATGACC TTGTCGACCGGTTATCGGTCCCCTGCTCCTTCGAGCTGGCAAGGCGCTCA CAGGTTCTAC
For_PEMV2	5'GCG TAA TAC GAC TCA CTA TAG GTA G
Rev_PEMV2_ _crystallization	5'mGmGT AGA ACC TGT GAG CGC CTT GCC
Rev_PEMV2_ _EMSA	5'mGmT AGA ACC TGT GAG CGC CTT GCC

REFERENCES

1. Lescoute,A. and Westhof,E. (2006) Topology of three-way junctions in folded RNAs. *Rna*, **12**, 83–93.