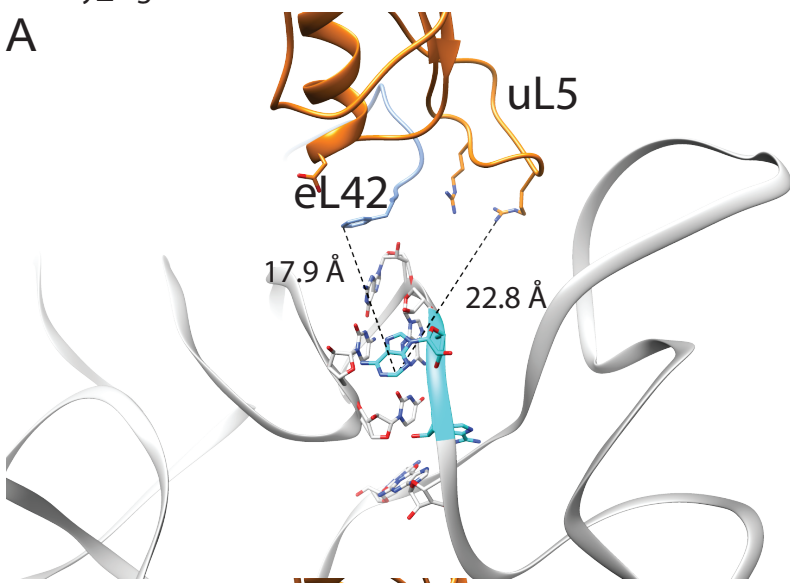
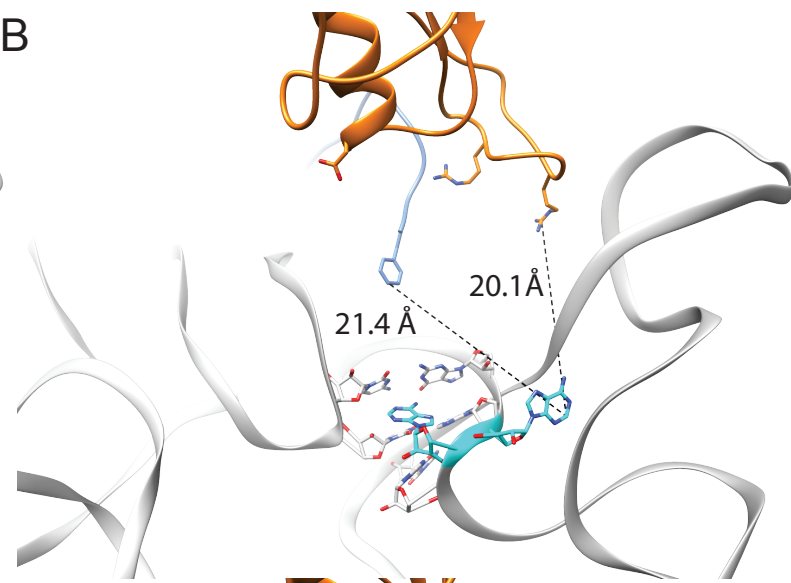


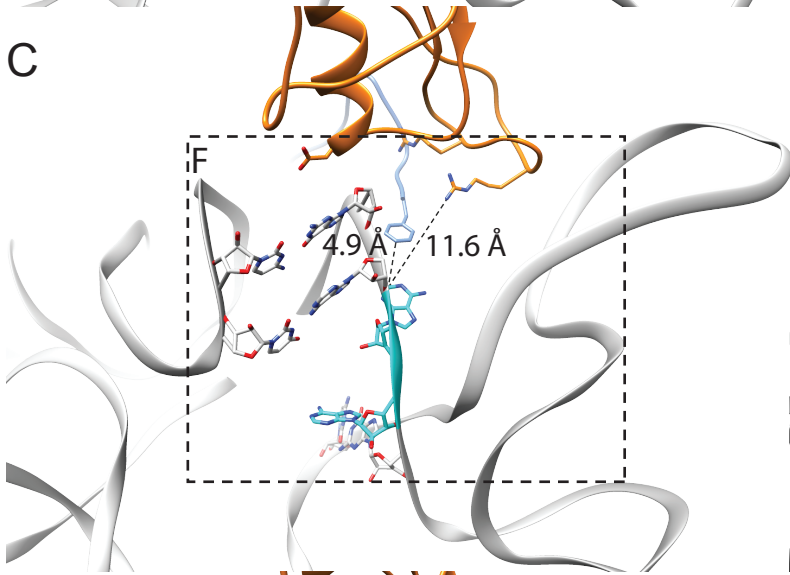
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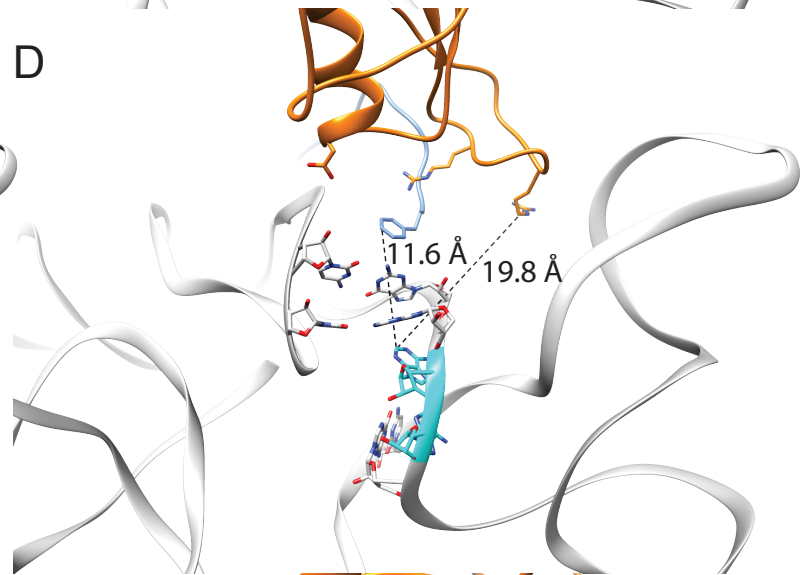
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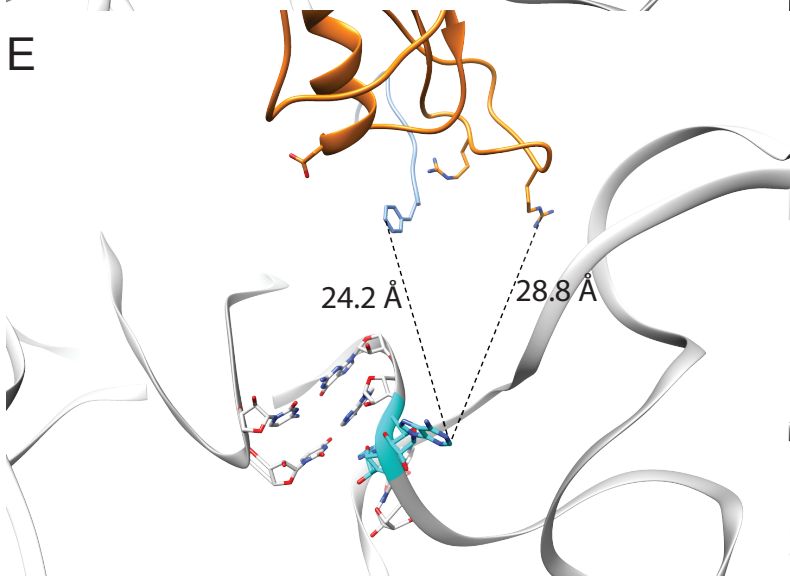
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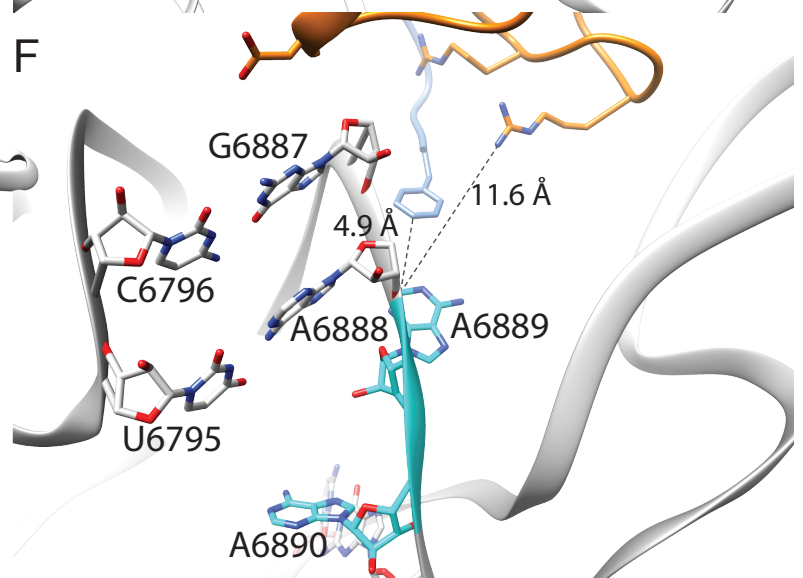
D



E



F



1 **Figure S1: Intermediate states of the TSV IRES involved in eEF2-mediated**
2 **pseudotranslocation.** The five states characterizing the inchworm like translocation
3 mechanism proposed for the TSV IRES as determined by cryo-EM are shown (A-E;
4 PDBs 5juo, 5jup, 5jus, 5jut, 5juu) (1). These illustrate a significant degree of structural
5 rearrangements in the hinge region to facilitate the conformation changes involved.
6 eL42 (blue) and uL5 (orange) are observed to approach the upstream region of the
7 hinge (nucleotides GA6886-7) but do not contact AA6889-90 directly, which instead
8 adopt different orientations that could be involved in mediating the specific interactions
9 required for translocation. A zoomed view of the state with the closest contact between
10 eL42/uL5 and the hinge is shown in F (boxed in C). Distances between A6889 and eL42
11 Phe106 or uL5 Arg55 are indicated. Also see Supplemental Movie 2.

12

13

14 **Movie S1: Morph between the IAPV IRES pre-translocation states shown in Figure**
15 **10.** Distances between U6547, A6548, A6546 and U6451 shown highlighting tight
16 packing around UA6547-8. The structures were superposed on nucleotides immediately
17 upstream of the hinge and a morph interpolating between the states was calculated
18 using Chimera (2). The morph loops forward then reverse between states to illustrate
19 conformational changes involved.

20

21 **Movie S2: Morph between the five TSV IRES states shown in Figure S1.** The
22 distances between A6889 and eL42 Phe106 or uL5 Arg55 are indicated. The structures
23 were superposed based on the uL5 structure and a morph interpolating between the

24 states was calculated using Chimera (2). The morph loops forward then reverse
25 between states to illustrate conformational changes involved.

26

27 REFERENCES

- 28 1. Abeyrathne PD, Koh CS, Grant T, Grigorieff N, Korostelev AA. 2016. Ensemble
29 cryo-EM uncovers inchworm-like translocation of a viral IRES through the
30 ribosome. *Elife* 5.
- 31 2. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC,
32 Ferrin TE. 2004. UCSF Chimera--a visualization system for exploratory research
33 and analysis. *J Comput Chem* 25:1605-12.

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