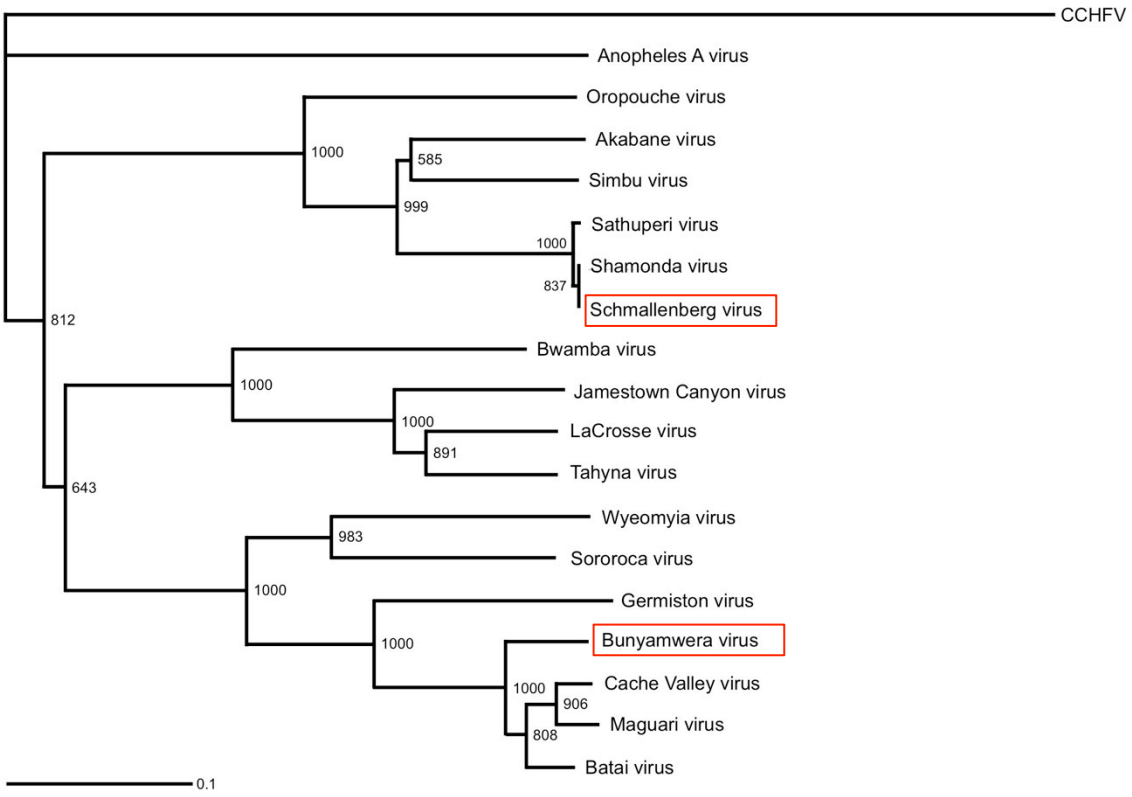

Figure S1. Phylogeny of bunyaviruses. Phylogenetic tree showing the evolutionary relationship between orthobunyaviruses based on N protein amino acid sequence alignments; SBV and BUNV are distantly related within the *Orthobunyavirus* genus, but the N proteins have almost identical structures. The two viruses used in this study are highlighted. The nairovirus CCHFV is included as an outlier to root the tree. Tree built using treeview [40].

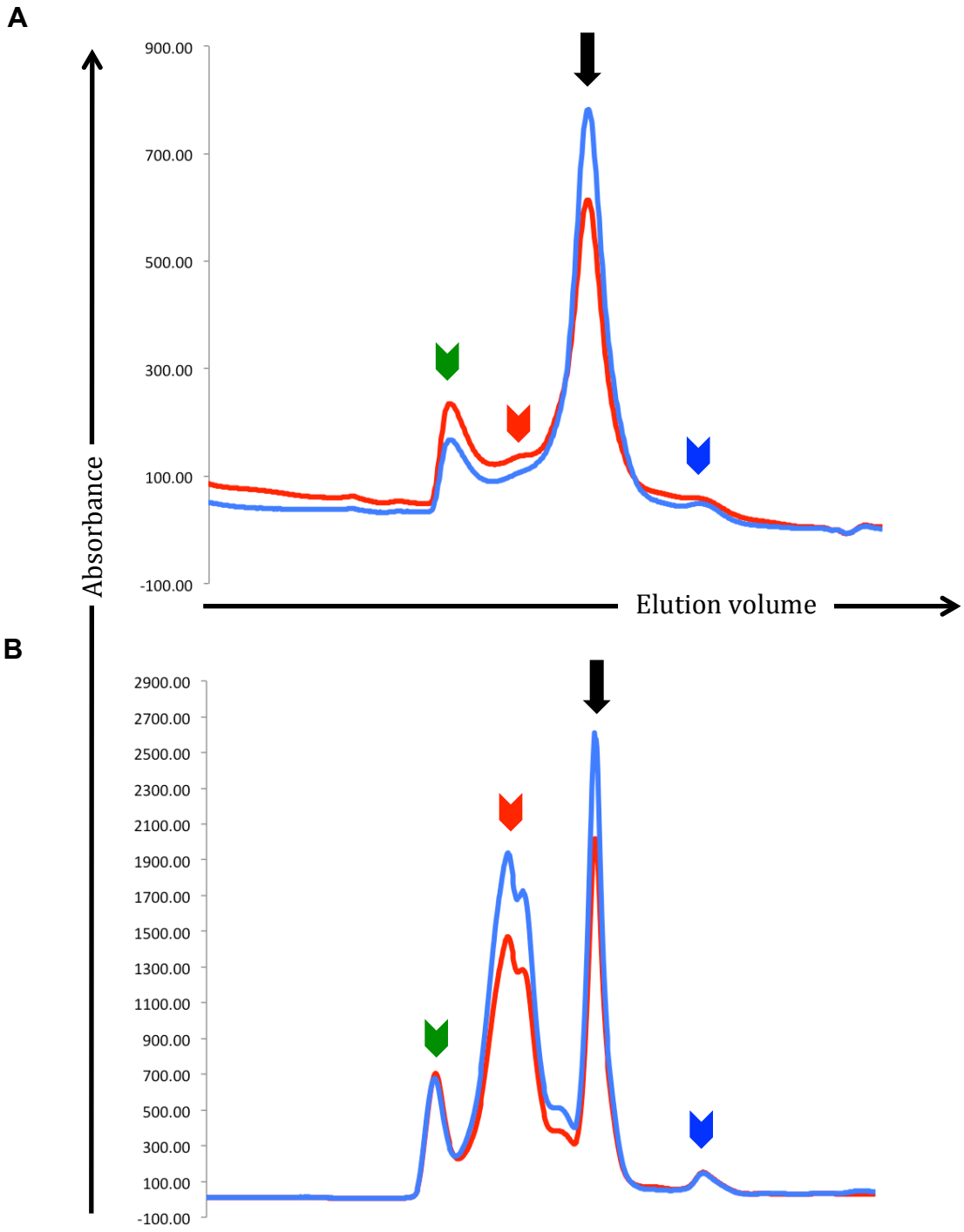

Figure S2. Gel Filtration chromatograms of purified apo BUNV and SBV N proteins (A) BUNV N (B) SBV N. Absorbance at 280 nm is shown in blue, 260 nm in red. Black arrows indicate the tetramer, red arrowheads higher order oligomers, green arrowheads large aggregates eluting at the void volume, blue arrowhead marks the peak for 6xHis-SUMO (cut from the fusion protein after initial purification on a nickel column)

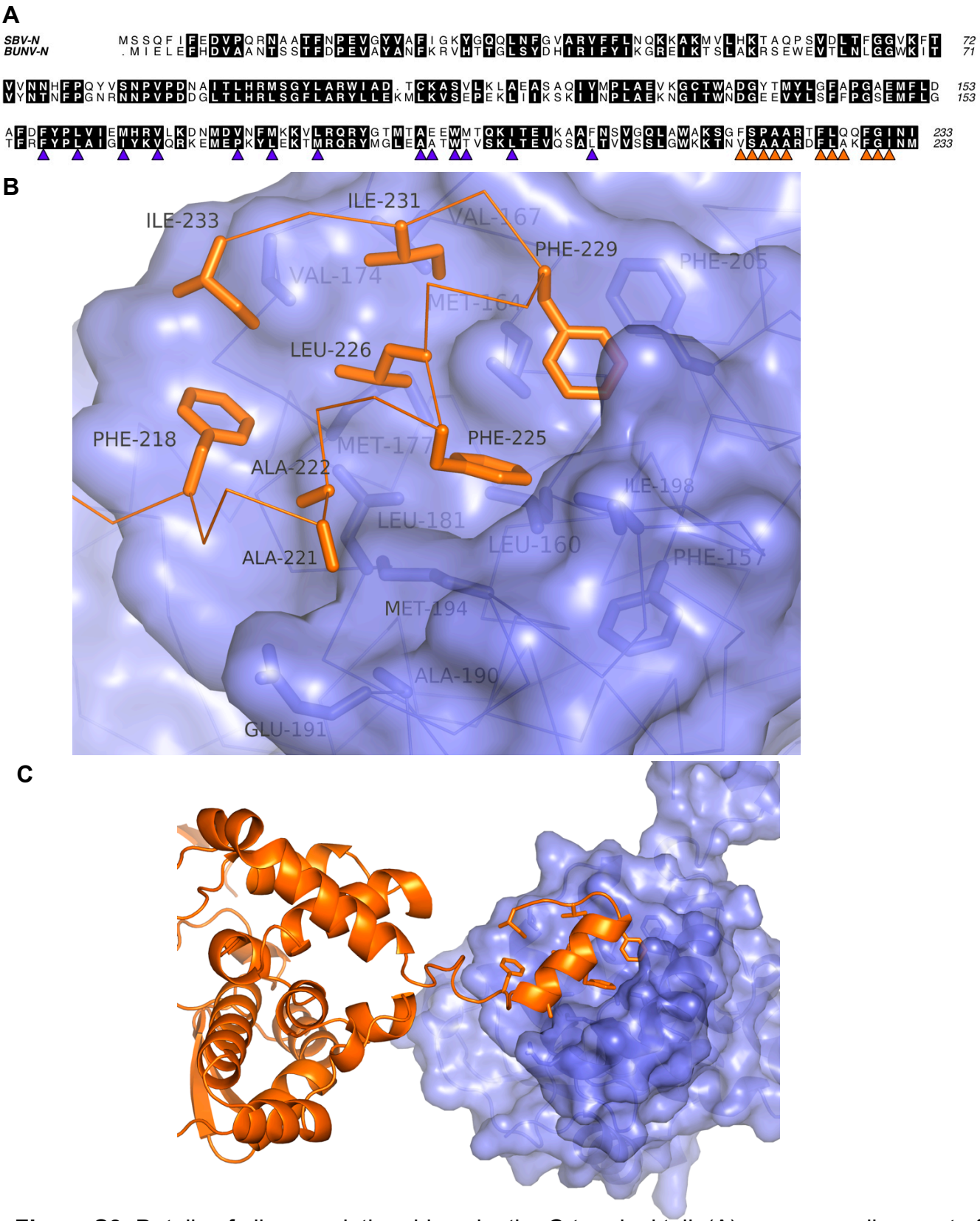

Figure S3. Details of oligomersiation driven by the C-terminal tail. (A) sequence alignment of SBV and BUNV N proteins highlighting similar amino acids in black. Blue triangles label residues making up the hydrophobic patch to which the C-terminal tail binds, orange triangles label hydrophobic residues in the C-terminal tail. (B) hydrophobics in the C-terminal tail of SBV N bound to the hydrophobic patch of a neighbouring molecule within the tetramer (C) Overview of this interaction in the SBV N structure. Four such interactions mediate tetramerisation.

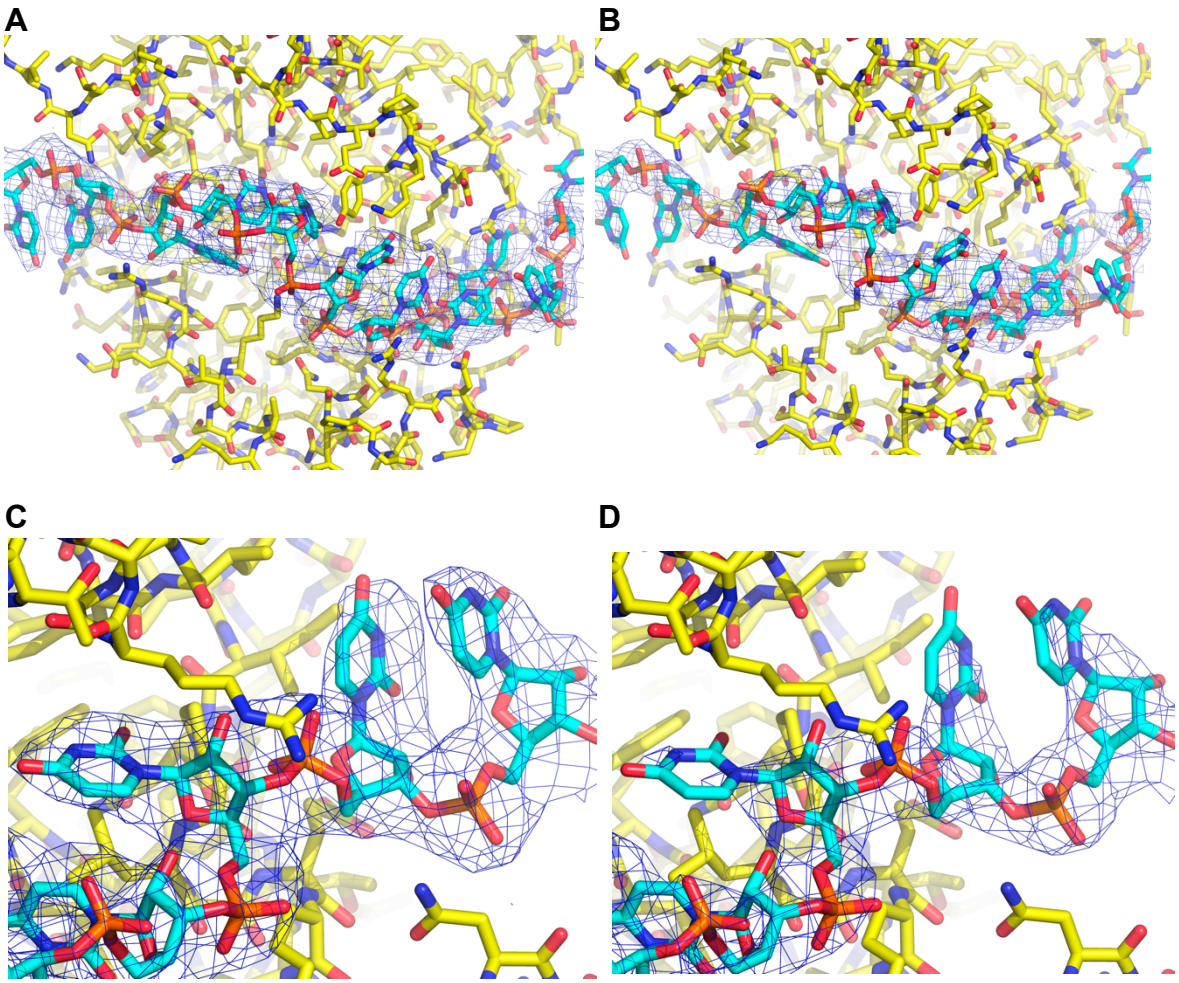

Figure S4. Electron density for RNA in the BUNV N + RNA structure. (A) Final refined 2Fo – Fc density for RNA only showing all RNA bound to a single monomer (B) Omit map density for the same RNA (C) Final refined 2Fo – Fc density zoomed in to show three bases (D) Omit map density for the same bases. The ribo-phosphate backbone for RNA is clearly visible even in omit map density, whereas the bases have weaker density due to the random sequences bound (originating from *E .coli* RNA).

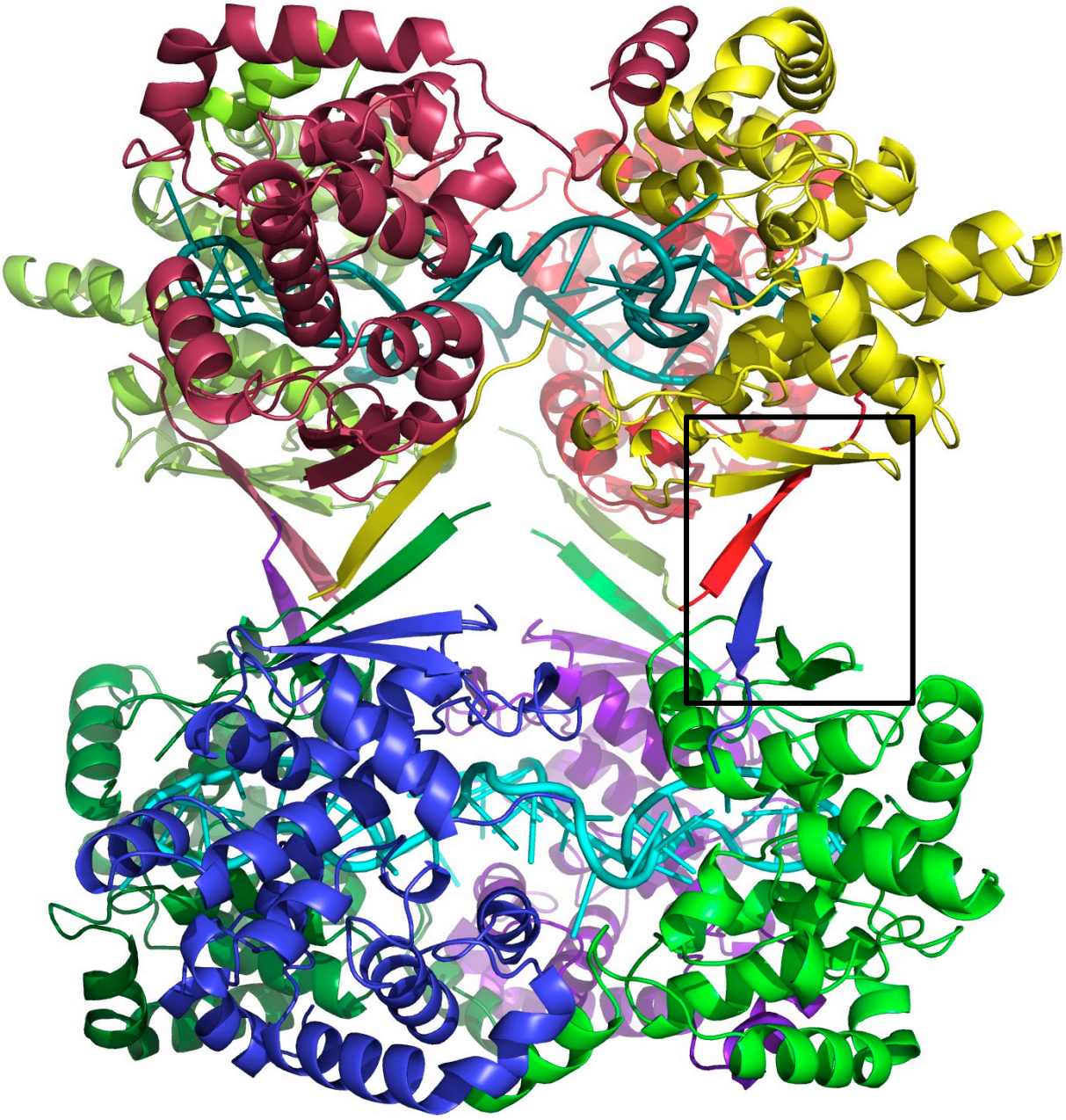

Figure S5. Details of N-terminal arm interactions. The N-terminal arm mediates formation of an octamer in crystals of BUNV N. The N-terminus of two molecules (coloured blue and red) forms a β-strand sitting between β-sheets of two other protomers (yellow and green) to form a 6-stranded β-sheet with contributions from 4 molecules (black box – strands coloured yellow, red, blue and green). Four such interactions mediate crystal contacts between tetramers in the BUNV crystals.

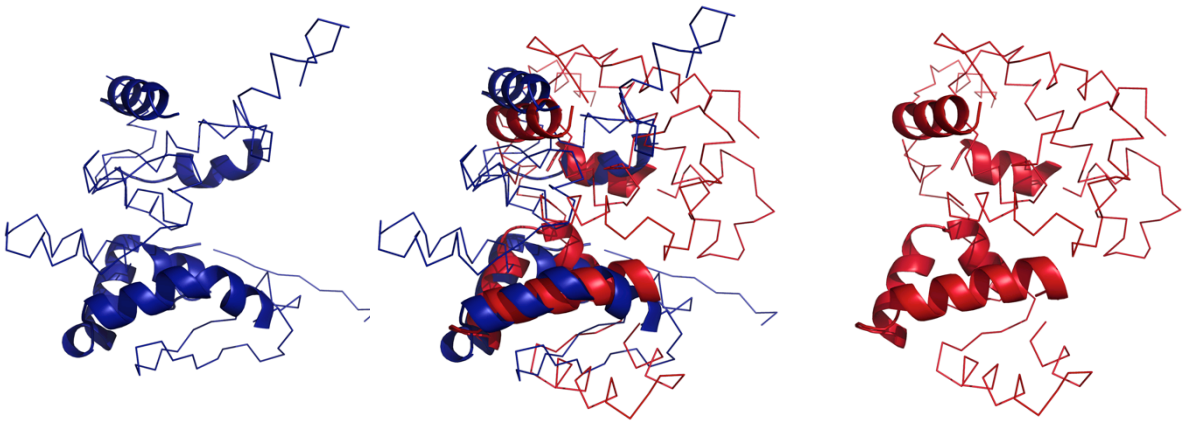

Figure S6. Structural alignment of N proteins from an orthobunyavirus (SBV – blue), and a phlebovirus (RVFV – red) reveals a limited structural similarity across these two bunyavirus genera; no such similarity is evident for the nairovirus CCHFV [16,18,19]; there are no crystal structures of N proteins from other bunyavirus genera tospoviruses or hantaviruses.

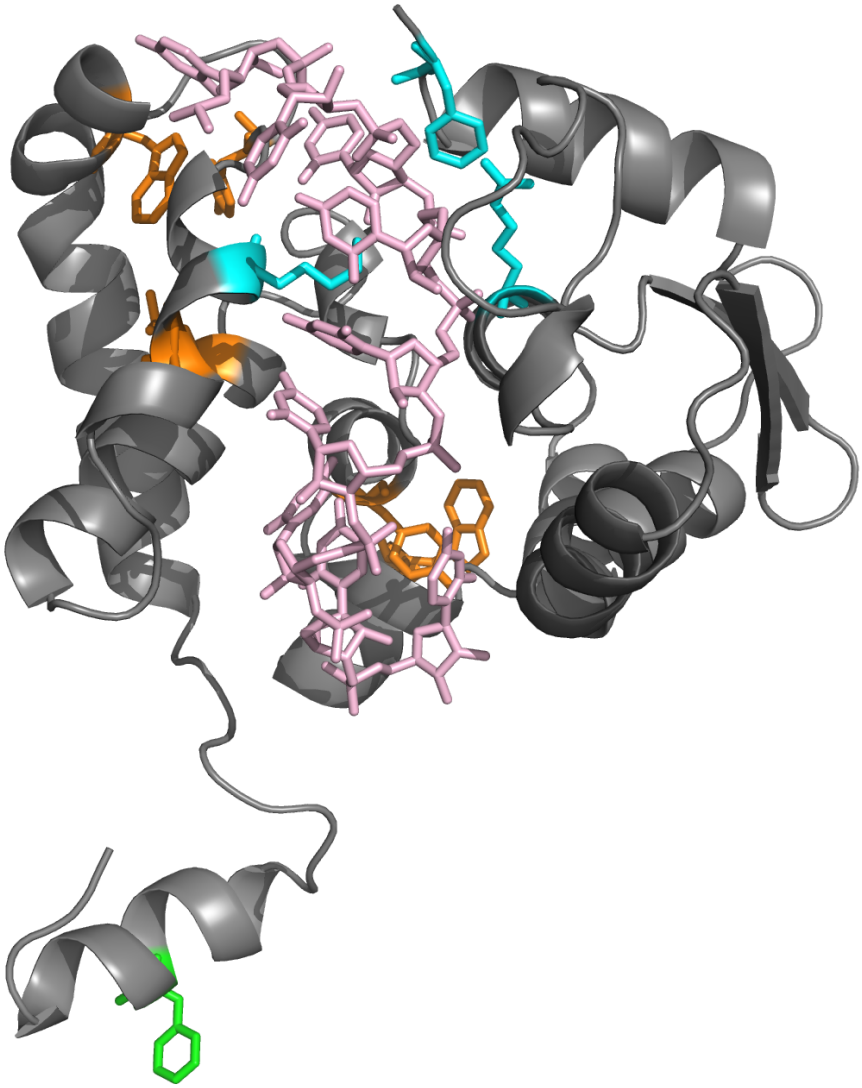

Figure S7. Structural interpretation of previously published mutagenesis data. Mutants of BUNV N defined as generating nonrescuable virus [44], or defective viral genomic RNA synthesis [45], are mapped on to our BUNV structure (grey) with bound RNA (pink). Residues highlighted in cyan (F17, R94, K179) are close to RNA within the binding groove, therefore likely to reduce RNA binding affinity when mutated. Residues in orange (W134, Y141, L160, L177, Y185, W193) are buried hydrophobic residues that could disrupt the N protein fold if mutated. Residue 225 highlighted in green is a phenylalanine within the C-terminal helix buried against a neighbouring molecule, thus its mutation would likely disrupt oligomerisation (similar to the ts19 mutant – see main text)

Supplementary References

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