Supplemental Material

Crystallographic and cryoEM analyses reveal SARS-CoV-2 SL5 is a mobile T-shaped four-way junction with deep pockets

Christopher P. Jones and Adrian R. Ferré-D'Amaré

Laboratory of Nucleic Acids, National Heart, Lung and Blood Institute, Bethesda, MD, USA

Address correspondence to C.P.J. (christopher.jones2@nih.gov) or A.R.F.

(adrian.ferre@nih.gov)

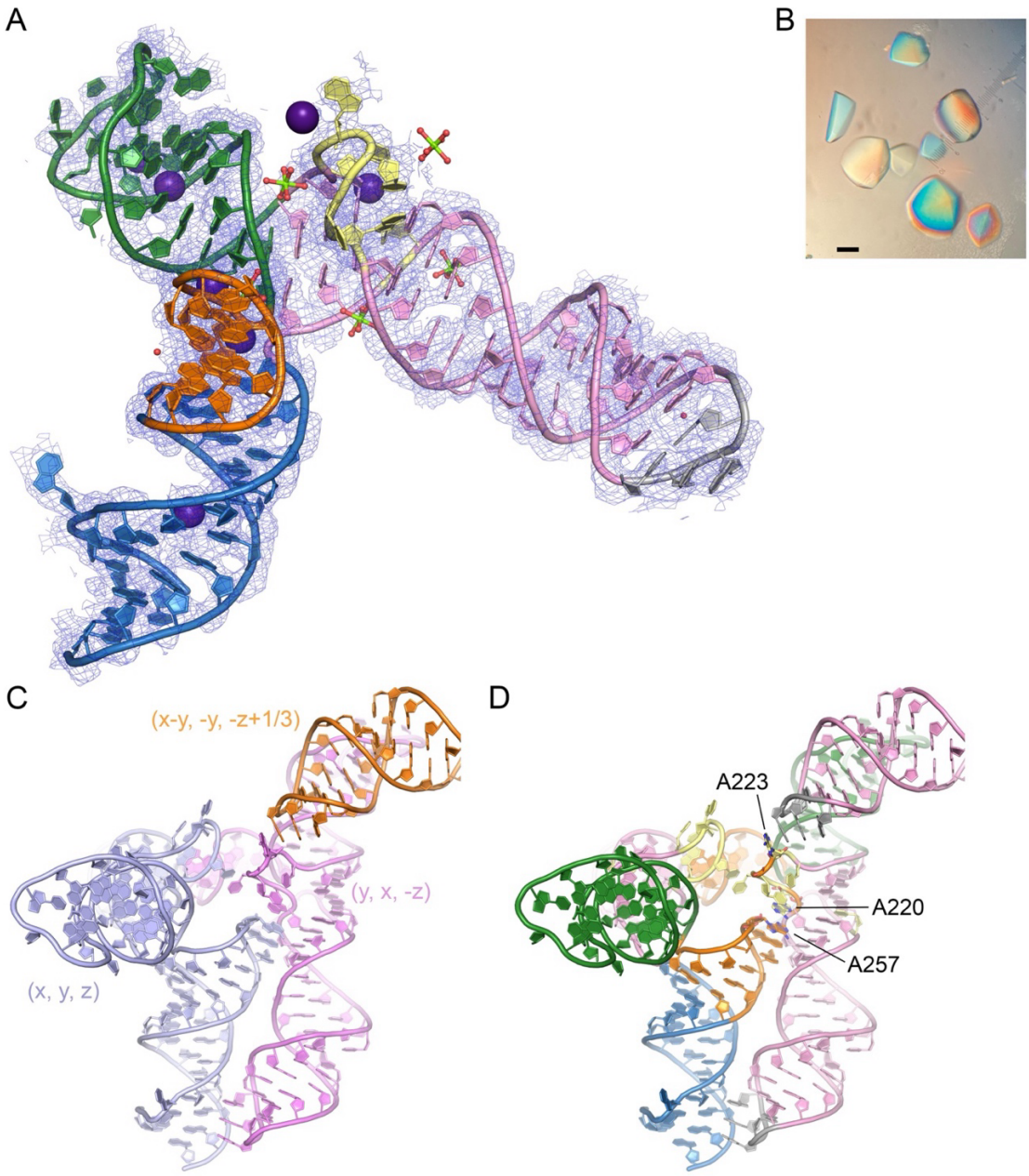

Fig. S1: Crystallization and solution of SARS-CoV-2 SL5 structure

A, Density-modified SAD experimental electron density map of SL5 from dataset 1, contoured at 1.5 σ (blue mesh) overlaid with the refined structure. Pink, purple, and lime spheres denote waters, Cs^{+} ions, and hydrated Mg^{2+} ions, respectively. **B**, Example crystals of SL5 in cryoprotectant solution. Scale bar, 100 µm. **C**, Crystal contacts from one copy of SL5 (lavender) with neighboring copies (magenta and orange). Symmetry operators are indicated. **D**, Same as **C** but with each symmetry-related molecule colored as in **A**. Crystal packing brings the SL5a bulge (yellow) of two copies into close proximity. Shown are interactions between A223 with the crystallization-promoting mutations (grey) of a symmetry-mate and crystal contacts between SL5c A257 and A220 of a symmetry-mate.

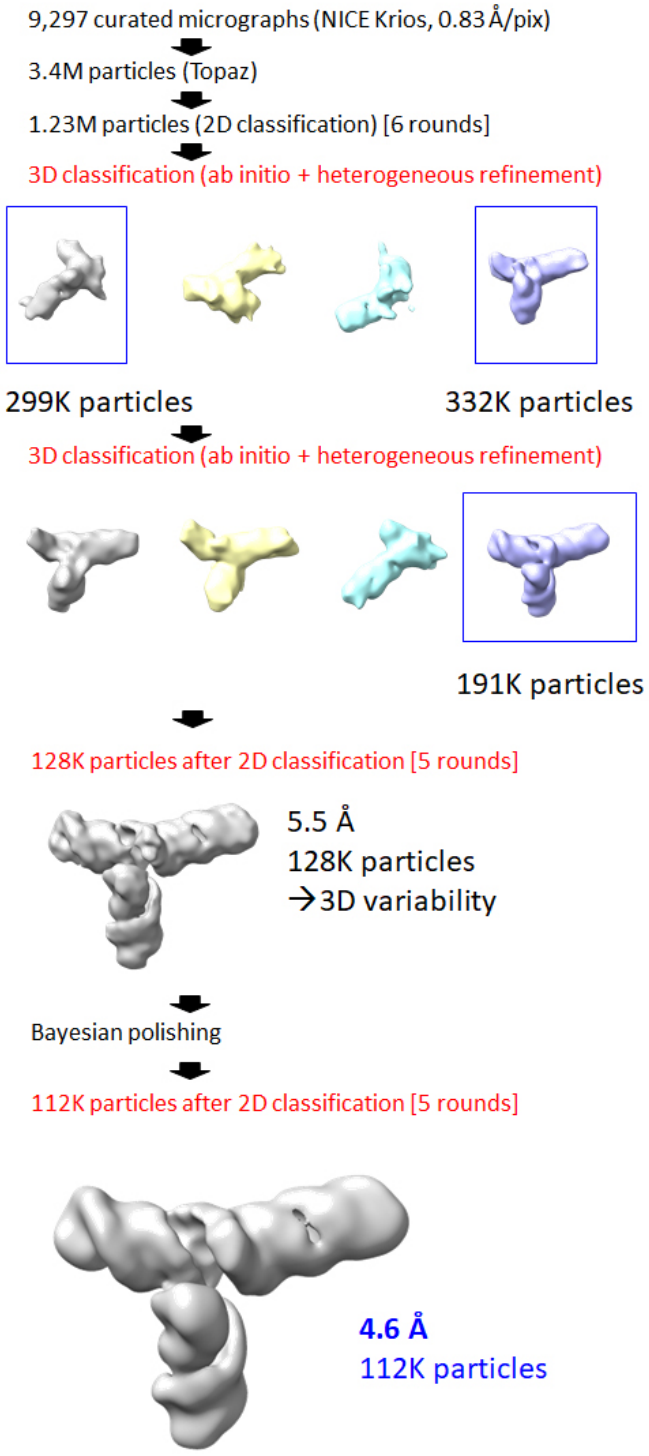

Fig. S2: Cryo-EM data analysis for SARS-CoV-2 SL5

Data-processing pipeline for SARS-CoV-2 SL5. Steps in black were performed in RELION, and steps in red were performed in cryoSPARC. Blue boxes indicate classes taken for subsequent steps, and the step at which 3D variability analysis was performed is indicated.

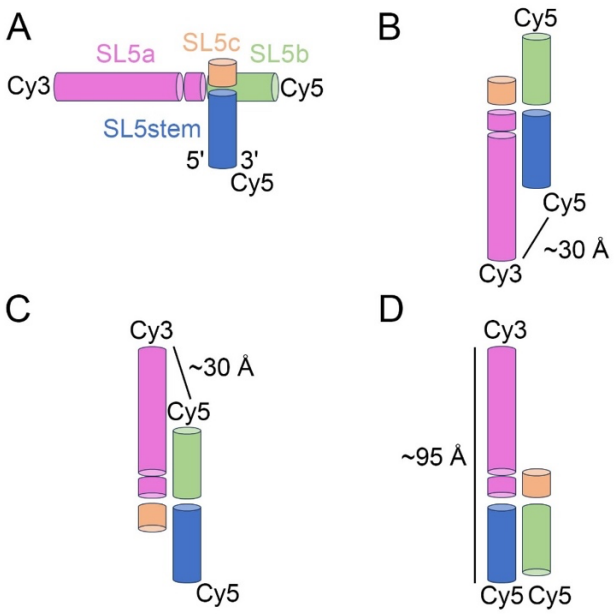

Fig. S3. **FRET labeling scheme to evaluate T-shaped and H-shaped SL5 folds**

A, Schematic of the crystallographically determined SL5 fold, with helices shown as cylinders and colored as in **Fig. 1**. Cy3 and Cy5 fluorophore locations are indicated. **B**, Alternative, hypothetical SL5 conformation in which SL5a and SL5stem termini are brought close to one another, bringing one Cy5 adjacent to Cy3. **C**, Second alternative, hypothetical SL5 conformation in which SL5a and SL5b termini are brought close. **D**, Third alternative, hypothetical conformation in which neither Cy5 dye is proximal to SL5a. Estimated distances assume stacking of helices.

Table S1: Bulk FRET measurements for SL5

	Without Mg^{2+}		With Mg^{2+}	
Construct	**Cy5 at C241**	**Cy5 at 3' end**	**Cy5 at C241**	**Cy5 at 3' end**
Rep1	0.264	0.328	0.339	0.352
Rep2	0.349	0.302	0.388	0.392
Rep3	0.423	0.330	0.450	0.408
Rep4	0.410	0.333	0.354	0.373
Rep5	0.296	0.305	0.362	0.393
Rep6	0.249	0.353	0.392	0.343
Mean E_{FRET} [a]	0.332 ± 0.074	0.325 ± 0.019	0.381 ± 0.039	0.377 ± 0.025
***R*, Å** [b]	67.4 ± 15.1	67.8 ± 4.0	65.1 ± 6.7	65.2 ± 4.4

[a] Values are reported as the mean and standard deviation of 6 independent experiments.

[b] Distances calculated using an R_0 of 60 Å.

Table S2: Oligonucleotides used in this work

Name	Sequence
UTRLuc FP	Ctcaagctatgcatcaagc
UTRLuc RP	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGTCATTCTCCTAAGAAGC
SL5-101 RP[1]	mCmUCTCCATCTTACCTTTC
Plasmid SL5-101	TAATACGACTCACTATAgggagaAGATAGACGAGctgatgagtccgtgaggacgaaacggtacccggtaccgtcCTCGTCTATCTTCTGCAGGCTGCTTACGGGAAACCGTGTTGCAGCCGATCATCAGCACATCTAGGTTTCGTCCGGGTGTGACCGAAAGGTAAGATGGAGAG
A266G	GTGTGACCGAAAGGTAAG**G**TGGAGAGCCTTGTCCCTG
U182C	GACACGAGTAACTCGTCTATCT**C**CTGCAGGCTGCTTACGG
A263G	CGGGTGTGACCGAAAGGT**G**AGATGGAGAGCCTTGTC
U262C	CGGGTGTGACCGAAAGG**C**AAGATGGAGAGCCTTGTC
A253G	GGTTTCGTCCGGGTGTG**G**CCGAAAGGTAAGATGGAG
A253G,U262C	GGTTTCGTCCGGGTGTG**G**CCGAAAGG**C**AAGATGGAGAGCCTTGTC
G256U,A257U,A258U	GGTTTCGTCCGGGTGTGACC**TTT**AGGTAAGATGGAGAGCCTTGTC
G252A	GGTTTCGTCCGGGTGT**A**ACCGAAAGGTAAGATGGAG
C228U	GTTGCAGCCGATCATCAG**T**ACATCTAGGTTTCGTCC
G227A	GTTGCAGCCGATCATCA**A**CACATCTAGGTTTCGTCC
C183U	CGAGTAACTCGTCTATCTT**T**TGCAGGCTGCTTACGG
A187C	CTCGTCTATCTTCTGC**C**GGCTGCTTACGGTTTC
C186A	AACTCGTCTATCTTCTG**A**AGGCTGCTTACGGTTTC
G219U	CGTCCGTGTTGCAGCC**T**ATCATCAGCACATCTAGG
G219C	CGTCCGTGTTGCAGCC**C**ATCATCAGCACATCTAGG
SL5a-GAAA	CTGCAGGCTGCTTACGG**GAAA**CCGTGTTGCAGCCGATC
ΔG174	CAGGACACGAGTAACTCTCTATCTTCTGCAGGC
ΔG211	CTTACGGTTTCGTCCGTGTGCAGCCGATCATCAG
ΔA235	CGATCATCAGCACATCTGGTTTCGTCCGGGTGTG
SL51-Cy3[2]	CUCGUCUAUCUUCUGCAGGCUGCUUACGGUUU**T**GUCCGUG
SL52	UUGCAGCCGAUCAUCAGCACAUCUAGGUUUCGUCCGGGUG
SL52-Cy5[3]	UUGCAGCCGAUCAUCAGCACAUCUAGGUUU**T**GUCCGGGUG
SL53[4]	UGACCGAAAGGUAAGAUGGAGAG
SL53-Cy5[4]	UGACCGAAAGGUAAGAUGGAGA**G**
DNAsplint	CCTTTCGGTCACACCCGGACGAAACCTAGATGTGCTGATGATCGGCTGCAACACGGACGAAACCGTAAGCAGCCTGCAG

[1] 2'-O-methylated positions indicated by "m".
[2] The bold underlined T residue indicates the position of a dT residue with a C6 linker attached to a Cy3 dye.
[3] The bold underlined T residue indicates the position of a dT residue with a C6 linker attached to a Cy5 dye.
[4] The bold underlined G residue indicates the position of 3' phosphate attached to a Cy5 dye.