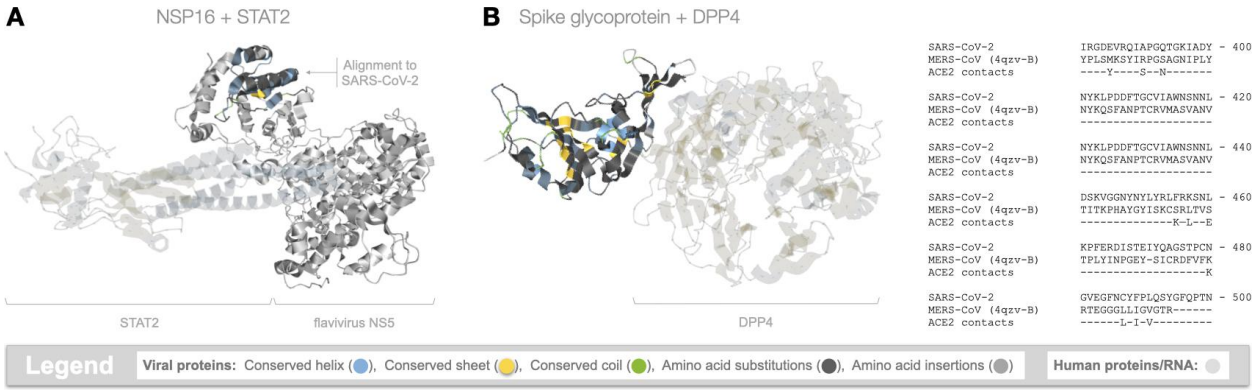


Appendix Figure S1. Assessing structural evidence for STAT2 and DPP4 hijacking

These matching structures show putative interactions between viral and human proteins, but detailed analysis finds insufficient evidence that these proteins are hijacked by SARS-CoV-2. The structures are shown with Aquaria's default coloring scheme which, for viral proteins, highlights secondary structure as well as any amino acid substitutions and insertions in the alignment the SARS-CoV-2 sequences; human proteins are rendered as semi-transparent.

A. Hijacking of STAT2 was shown in only one matching structure that was determined using a distantly related flavivirus protein called NS5 (Wang et al., 2020). Mapping the SARS-CoV-2 NSP16 sequence onto this structure (P0DTD1/6wcz) reveals that the alignment has moderate significance ($E = 10^{-16}$), low pairwise identity (13%), and does not include any of the residues that contact STAT2. Thus, we conclude that this matching structure does not provide evidence of hijacking of STAT2 by SARS-CoV-2.

B. Hijacking of DPP4 was shown in only two matching structures, both determined using the spike glycoprotein from MERS-CoV (Lu et al., 2013; Wang et al., 2014). Mapping the SARS-CoV-2 spike glycoprotein sequence onto these structures reveals that the alignment has high significance ($E \sim 10^{-46}$); however, for the structure shown here (P0DTC2/4qzv), the overall pairwise similarity is low (21%), with no sequence identities amongst the 10 residue positions that directly contact DPP4. Thus, we concluded that these matching structures do not provide strong evidence of hijacking of STAT2 by SARS-CoV-2.

Made using Aquaria and edited in Keynote.

References

Lu, G., Hu, Y., Wang, Q., Qi, J., Gao, F., Li, Y., Zhang, Y., Zhang, W., Yuan, Y., Bao, J., Zhang, B., Shi, Y., Yan, J., Gao, G.F., 2013. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. Nature 500, 227–231. https://doi.org/10.1038/nature12328

Wang, B., Thurmond, S., Zhou, K., Sánchez-Aparicio, M.T., Fang, J., Lu, J., Gao, L., Ren, W., Cui, Y., Veit, E.C., Hong, H., Evans, M.J., O'Leary, S.E., García-Sastre, A., Zhou, Z.H., Hai, R., Song, J., 2020. Structural basis for STAT2 suppression by flavivirus NS5. Nat. Struct. Mol. Biol. 27, 875–885. https://doi.org/10.1038/s41594-020-0472-y

Wang, Q., Qi, J., Yuan, Y., Xuan, Y., Han, P., Wan, Y., Ji, W., Li, Y., Wu, Y., Wang, J., Iwamoto, A., Woo, P.C.Y., Yuen, K.-Y., Yan, J., Lu, G., Gao, G.F., 2014. Bat Origins of MERS-CoV Supported by Bat Coronavirus HKU4 Usage of Human Receptor CD26. Cell Host Microbe 16, 328–337. https://doi.org/10.1016/j.chom.2014.08.009

1 1,000 2,000 3,000 4,000

Polyprotein 1a (PP1a)

NSP1 NSP2 NSP3 NSP4 NSP5 NSP6 7 NSP8 9 10

4 NTR

14 CTR

14 40S, 43S, or 80S

4 Ubl1

399 Macro

mimics

2 MACROD1

7 MACROH2A1

1 PARP9

1 GDAP2

4 MACROD2

2 MACROH2A2

25 PARP14

1 SUD-M

1 SUD-N

3 SUD-C

hijacks

1 PAIP1

65 PL-Pro

hijacks

5 ISG15

2 UBA52

3 UBB

2 UBC

1

3 NAB

4 NSP4C

binds

2 Inhibitory peptides

450 3CL-Pro

binds

1

42 Inhibitory peptides

35 34 16

1 HLA

32

1 HLA

21 NSP12

1 NSP12

15 vRNA

4 NSP13

1

4 NSP14

38 NSP16

47

Team 1 (vRTC)

Team 2

4 vRNA

5,000 6,000 7,000

PP1a

Polyprotein 1b (PP1b)

Spike glycoprotein

NSP12 / RdRp Polymerase NSP13 / Helicase NSP14 NSP15 NSP16

168 128 4 33 94

53 vRNA

22 NSP8

21 NSP7

15 vRNA

4

1 NSP9

Team 1 (vRTC)

binds

1 HLA

3 vRNA

mimics

13 AQR

12 spliceosome

9 UPF1

1 UPF2

2 IGHMBP2

1 hRNA

binds

4 NSP10

Team 2 (NSP10,14,16)

1 vRNA

binds

38 NSP10

4 vRNA

mimics

3 CMTR1

1 hRNA

1 MRM2

1,273 275 419

PP1b

Spike glycoprotein ORF3a E M 7a 8 N protein 9b

6 7b 9c 10

474 4 4 34 4

2 2 22 hijacks

N-NTD

N-CTD

hijacks

46 ACE2

2 SLC6A19

1 NRP1

binds

224 Antibodies

9 Inhibitory peptides

hijacks

1 APOA1

1 MPP5

binds

1 Antibody

2 HLA

2 vRNA

4 HLA

1 TOMM70

Legend: SARS-CoV-2 structural coverage map

Known structure

Viral protein sequence

Unknown structure

4 Number of structures

Substitutions

Helix Coil Sheet — Conserved residues

Indicates this is part of a much larger structure

Other proteins or RNA

XYZ Name of protein domain

hijacks — Structures show viral proteins hijacking human proteins

mimics — Structures show human proteins that the viral protein may mimic

binds — Structures show binding to viral proteins, HLA, or inhibitory peptides

40 Viral protein A — 40 structures show binding to viral protein A

8 Viral protein B — Of the above 40 structures, eight show binding to viral protein B

1 — One structure shows binding between the two adjacent proteins

Abbreviations:

40S: small ribosomal subunit

43S: pre-initiation complex

80S: ribosome

E: viral envelope protein

HLA: human leukocyte antigen

hRNA: host RNA

M: matrix glycoprotein

N: nucleocapsid protein

vRNA: viral RNA

vRTC: viral replication and translation complex

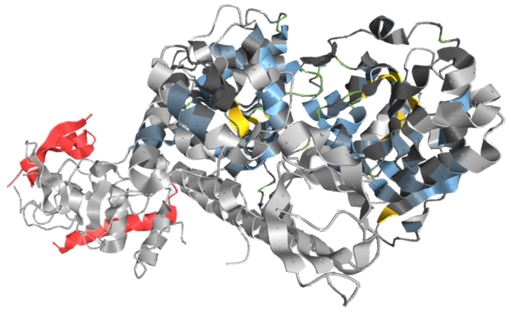
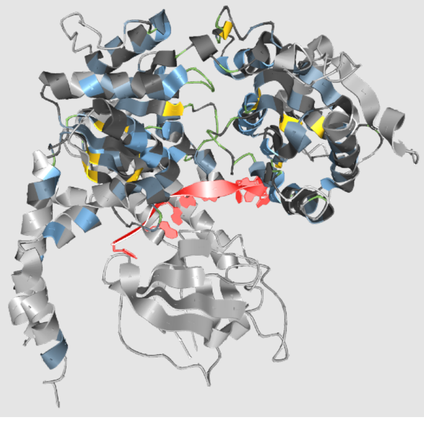
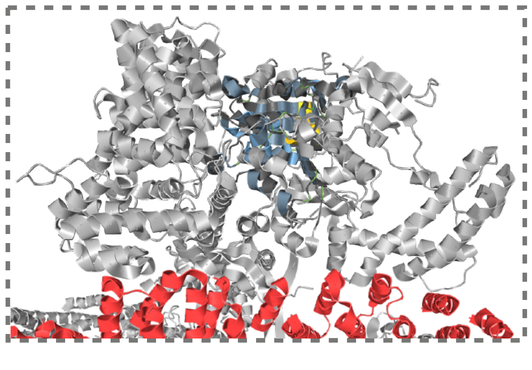
Data sources:

- The coverage map was based on Swiss-Prot sequences available on March 11, 2021.
- These sequence have been aligned onto all related PDB structures available on March 27, 2021.
- A total of 2,060 related structures were found.
- 1,180 of these structures were found from database cross-references in PDB entries.
- An additional 880 related structural models were found using HHBlits with cut-offs of either $E \leq 10^{-10}$ or pairwise identity ≥ 90%.

A NSP3 Mimicry of Host Proteins

Host Protein	NSP3 Alignment E value	NSP3 Alignment Identity	Domain Topology (Aligned Domains)	Potentially Hijacked Function
MACROD2	10^{-17}	28%	1 — Macro domain (5051) — 425	Erases ADPr, PAR, and AAR from D and E amino acids
MACROD1	10^{-16}	27%	1 — Macro domain (5051) — 325	Erases ADPr, PAR, and AAR from D and E amino acids
GDAP2	10^{-15}	20%	1 — Macro domain (483) — CRAL-TRIO domain — 497	Binds ADPr; Transports lipids between membranes
MACROH2A1	10^{-13}	19%	1 — Histone subunit A — Macro domain (4935) — 372	Binds ADPr; Modifies chromatin state
MACROH2A2	10^{-12}	18%	1 — Histone subunit A — Macro domain (4935) — 372	Binds ADPr, PAR, and AAR; Modifies chromatin state
PARP14	10^{-12}	25%	1 — 783 — Macro domain (5051)	
	10^{-11}	20%	Macro domain (4917)	
	10^{-10}	19%	Macro domain (4911) — PARP catalytic domain — 1,801	Binds ADPr; Adds PAR to D and E amino acids
PARP9	*No 3D structure*		1 — Macro domain (5051)	
	10^{-11}	23%	Macro domain (260) — PARP catalytic domain — 854	Binds ADPr; Adds PAR to D and E amino acids

B NSP13 Mimicry of Host Proteins

Host Protein	NSP13 Alignment E value	NSP13 Alignment Identity	Sequence-to-Structure Alignment	3D Structure	Color Legend	Potentially Hijacked Function
UPF1	10^{-53}	24%	*NSP13 sequence aligned onto 3D structure of UPF1 bound to UPF2*		UPF2 Amino acid substitution Conserved residue: Helix Conserved residue: Sheet Conserved residue: Coil Does not align to NSP13	Binds zinc Unwinds RNA Hydrolyzes ATP Binds chromatin Binds telomeric DNA Regulates nonsense-mediated decay of mRNAs
IGHMBP2	10^{-32}	25%	*NSP13 sequence aligned onto 3D structure of IGHMBP2 bound to human RNA*		hRNA Amino acid substitution Conserved residue: Helix Conserved residue: Sheet Conserved residue: Coil Does not align to NSP13	Binds zinc Hydrolyzes ATP Unwinds RNA & DNA Repairs & recombines DNA Regulates transcription & translation Binds immunoglobulin mu chain switch region of genome
AQR	10^{-27}	19%	*NSP13 sequence aligned onto 3D structure of AQR bound to a spliceosome*		Spliceosome Amino acid substitution Conserved residue: Helix Conserved residue: Sheet Conserved residue: Coil Does not align to NSP13	Unwinds RNA Splices mRNA Hydrolyzes ATP Repairs DNA damage DNA synthesis and ligation

A NSP1 (CTR) + 40S ribosomal subunit

B NSP3 (SUD-N) + PAIP1

C NSP3 (PL-Pro) + ubiquitin-like domains

Ubiquitin-like domains: UBB UBC

D Spike glycoprotein + ACE2 + SLC6A19

ACE2

SLC6A19 SLC6A19

E Spike glycoprotein + NRP1

F Envelope protein + MPP5

G ORF9b protein + TOMM70

Legend

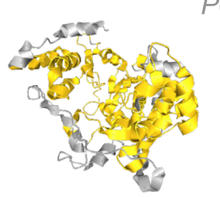
Viral proteins: Conserved helix (●), Conserved sheet (●), Conserved coil (●), Amino acid substitutions (●)

Human proteins/RNA: ●

A Viral interaction team 1 (NSP7, NSP8, NSP9, NSP12, NSP13)

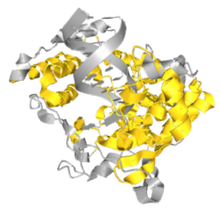
NSP12

P0DTD1/3n6m



NSP12 + RNA

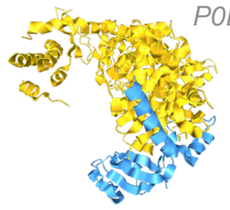
P0DTD1/5f8h



NSP8 (1)

NSP8 (1)

P0DTD1/6nus



No matching structures with NSP8 (1) + NSP12 + RNA

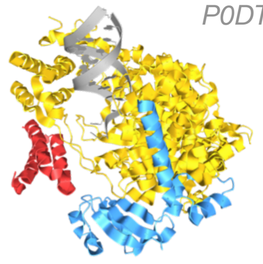
NSP7

P0DTD1/2kys

NSP7

NSP7

P0DTD1/7bv2



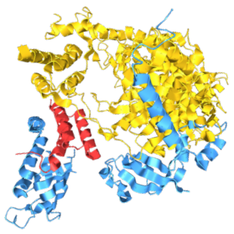
No matching structures with NSP7 + NSP8 (1) + NSP12

NSP8 (2)

P0DTD1/6wtc

NSP8 (2)

P0DTD1/7btf



NSP8 (2)

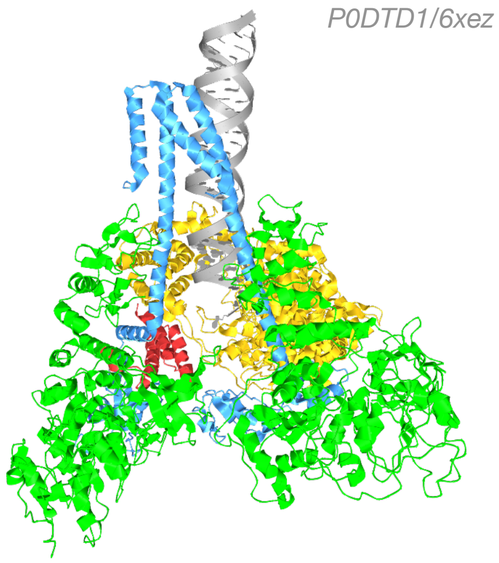
P0DTD1/6yyt

NSP13

NSP13

NSP13

P0DTD1/6xez



No matching structures with NSP7 + NSP8 (1) + NSP13

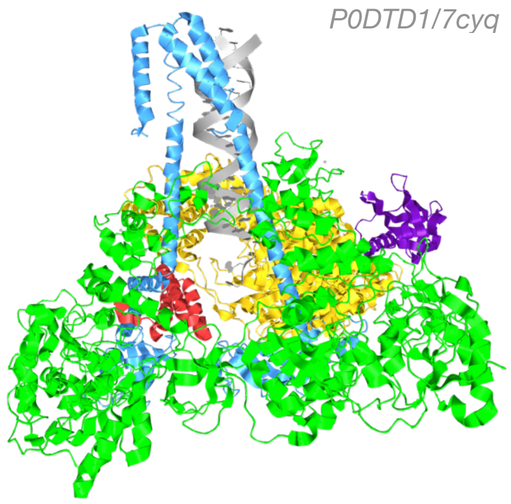
No matching structures with NSP7 + NSP8 (1) + NSP12 + NSP13

NSP9

NSP9

NSP9

P0DTD1/7cyq



No matching structures with NSP7 + NSP8 (1) + NSP9 + NSP13

No matching structures with NSP7 + NSP8 (1) + NSP9 + NSP12 + NSP13

RNA

RNA

RNA

RNA

NSP8 (1) + NSP12

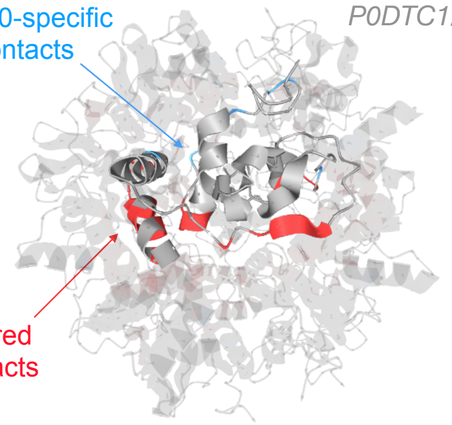
NSP8 (1) + NSP12

NSP8 (1) + NSP12

B Viral interaction team 2 (NSP10, NSP14, NSP16)

NSP10-specific contacts

P0DTC1/2ga6

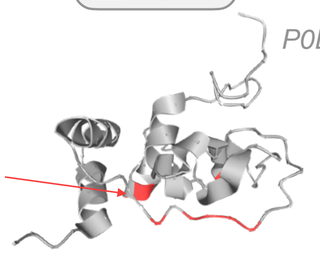


Shared contacts

NSP10 dodecamer

NSP10

P0DTC1/2fyg

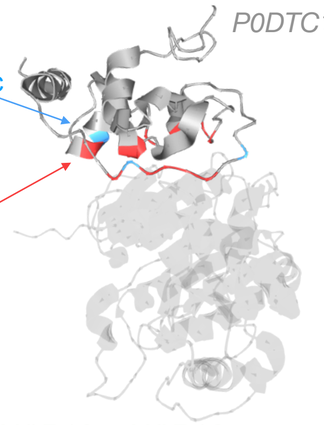


Common contacts

NSP10 monomer

NSP16

P0DTC1/6w61



NSP16-specific contacts

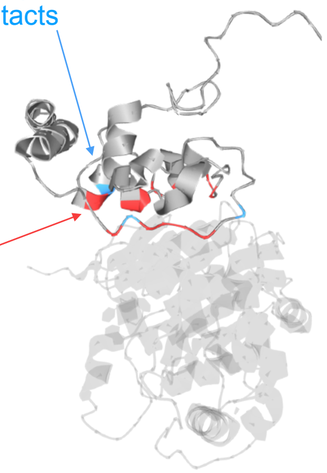
Shared contacts

NSP10 + NSP16

RNA

NSP16-specific contacts

P0DTC1/7jyy

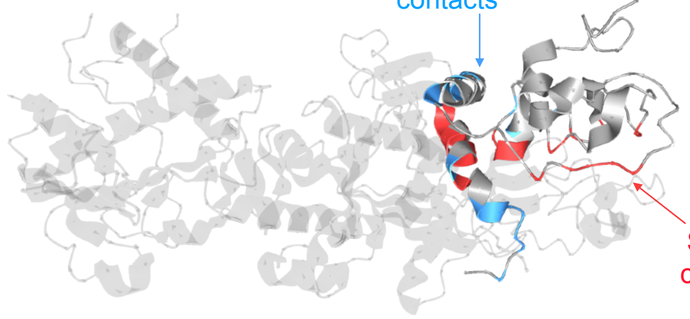


Shared contacts

NSP10 + NSP16 + RNA

NSP14

P0DTC1/5c8u



NSP14-specific contacts

Shared contacts

NSP10+NSP14