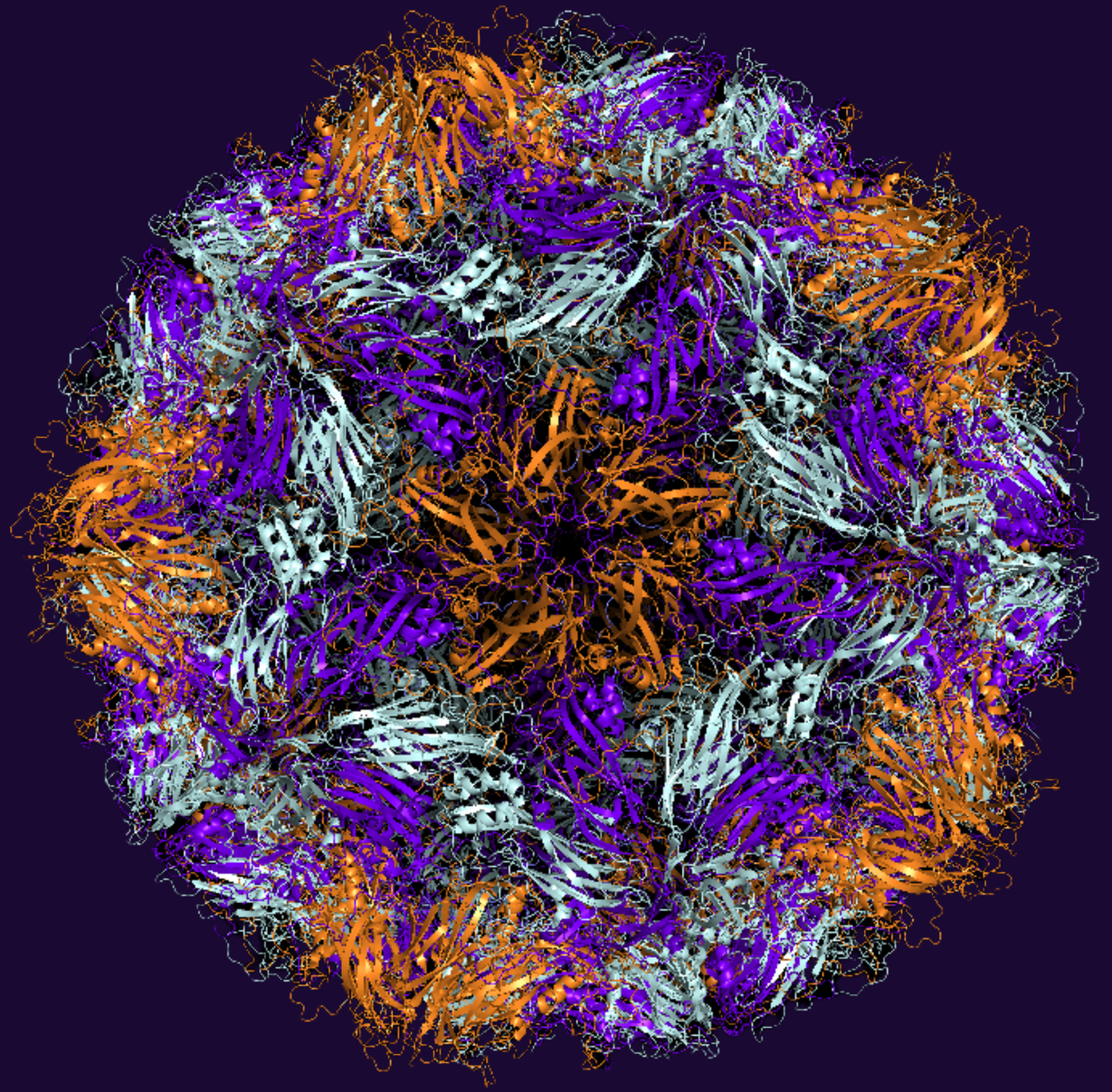


Picornavirus Capsid

Chloé Iniesta, Miguel Pérez and Mireia Sánchez

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Viral infection

Canyon
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Conclusions

The Family Picornaviridae

One of the most relevant viral families according to their impact on human and animal health

63 GENERA

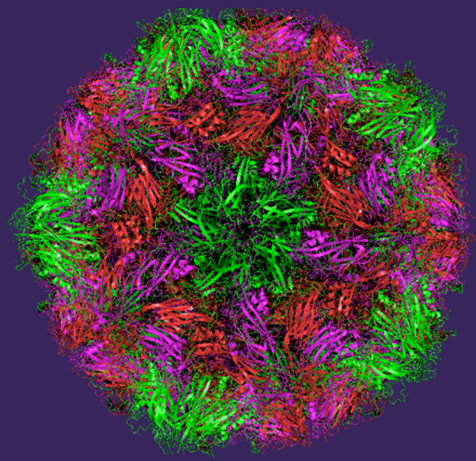
- **Enterovirus**
- Hepatovirus
- Cardiovirus
- 60 other genera

147 SPECIES

- **Rhinovirus B**
- Hepatitis A virus
- Theilovirus
- 144 other species

Non enveloped particles

- Capsid with icosahedral symmetry
- Single-stranded, positive-sense genomic RNA (~7-9 kb)



30nm in diameter

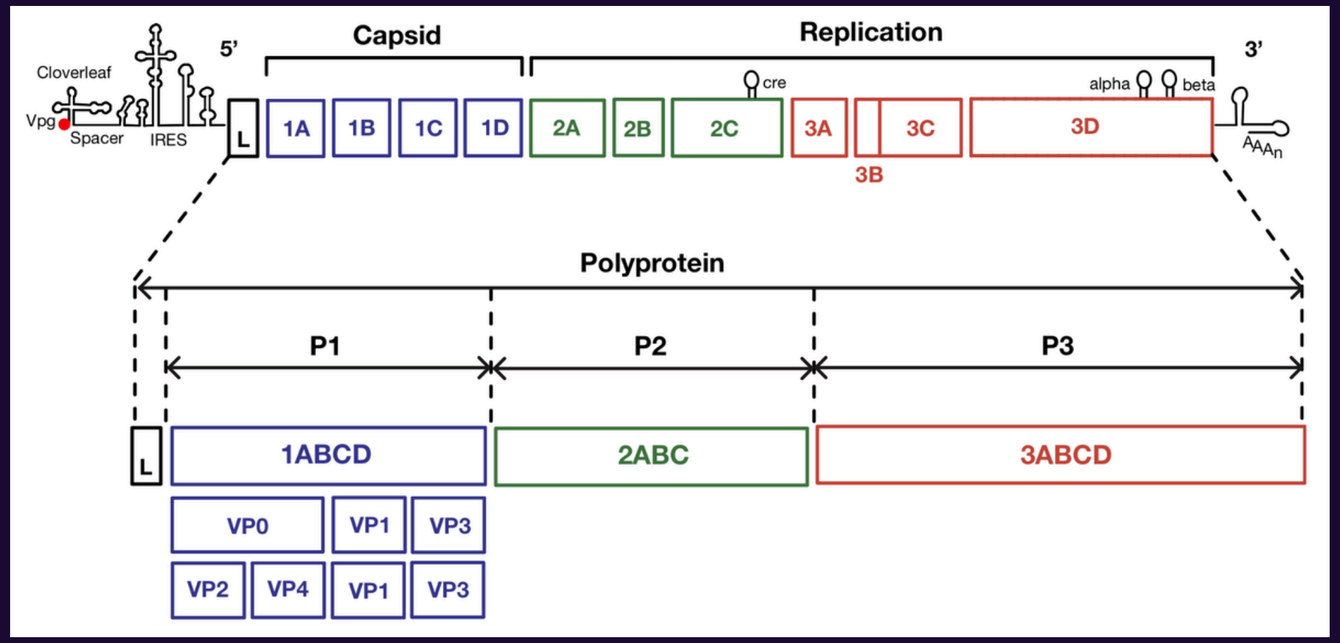
Family: Picornaviridae | ICTV

4RHV
Resolution: 3.00 Å

The Family Picornaviridae

Genome organization

Their RNA contains a single open reading frame (ORF) that encodes a polyprotein precursor for all viral proteins.



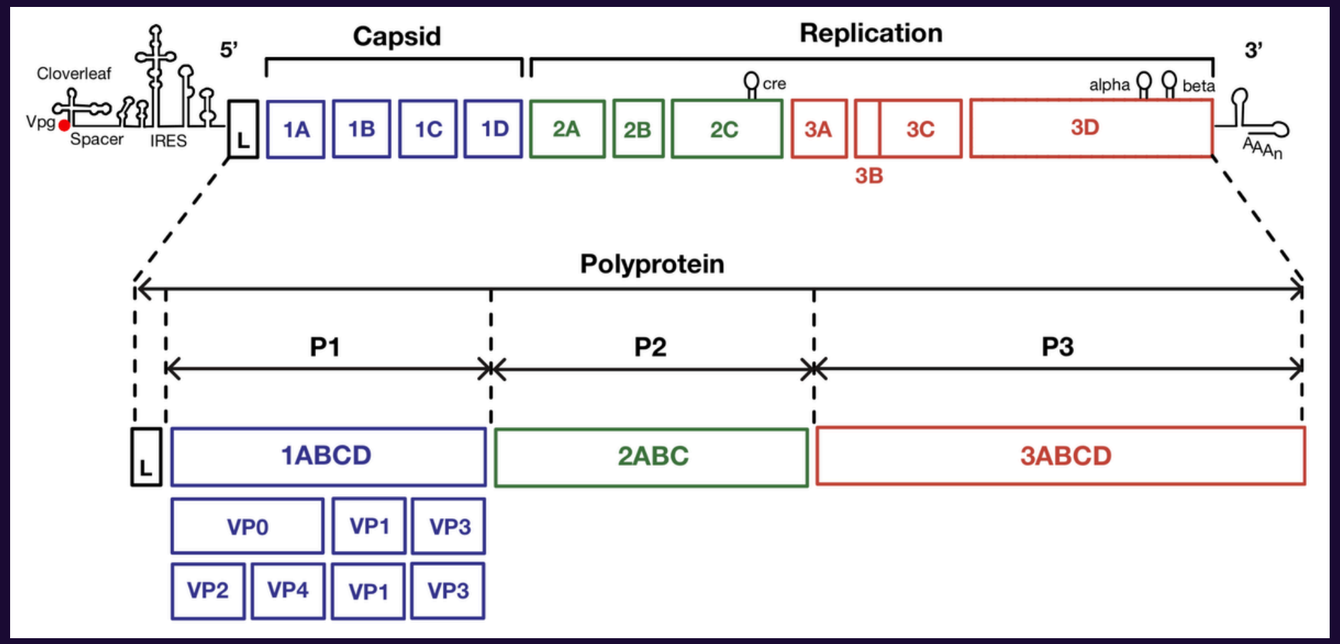
Adapted from: Picornavirus Morphogenesis - PMC

- **5' end**: RNA is covalently linked to the viral peptide VPg → replication and translation.
- **5' NTR**: "cloverleaf" and IRES.
- **ORF:** single large ORF.
- **3' NTR:** poly(A) tail → stabilization.

The Family Picornaviridae

Genome organization

Their RNA contains a single open reading frame (ORF) that encodes a polyprotein precursor for all viral proteins.



Adapted from: Picornavirus Morphogenesis - PMC

The ORF is divided into 3 units:

1ABCD - 2ABC - 3ABCD

- The numbers correspond to the three different functional domains
- The letters represent the proteins associated with each domain

P1 region: Encodes the structural proteins of the viral capsid.

P2 and P3 regions: Encode the non-structural proteins involved in viral replication.

Rhinovirus 14

Targets the upper respiratory tract ⟶ Causing common cold symptoms

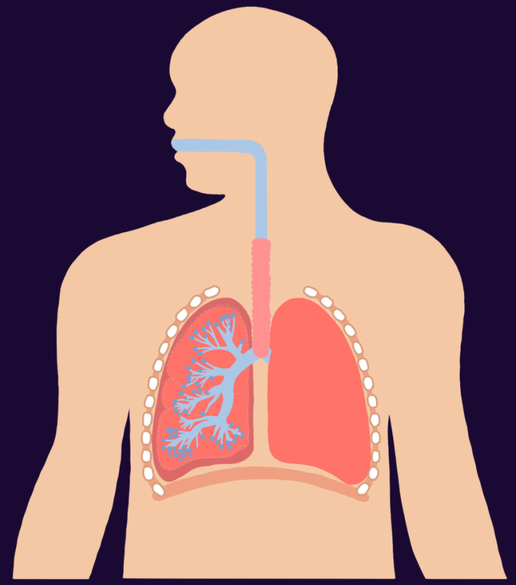
RNA genome is packed within a protein coat consisting of 4 viral proteins

- VP1
- VP2
- VP3

} Virus antigenic diversity

- VP4 ⟶ Anchors RNA to the capsid

There are 60 copies of each of the four capsid proteins, giving the virion an icosahedral structure, with a canyon in VP1 that serves as the site of attachment to cell surface receptors.



Replication → at 33°C optimally

Transmission → from person to person via contact or aerosols

The most important virus producing common cold syndrome

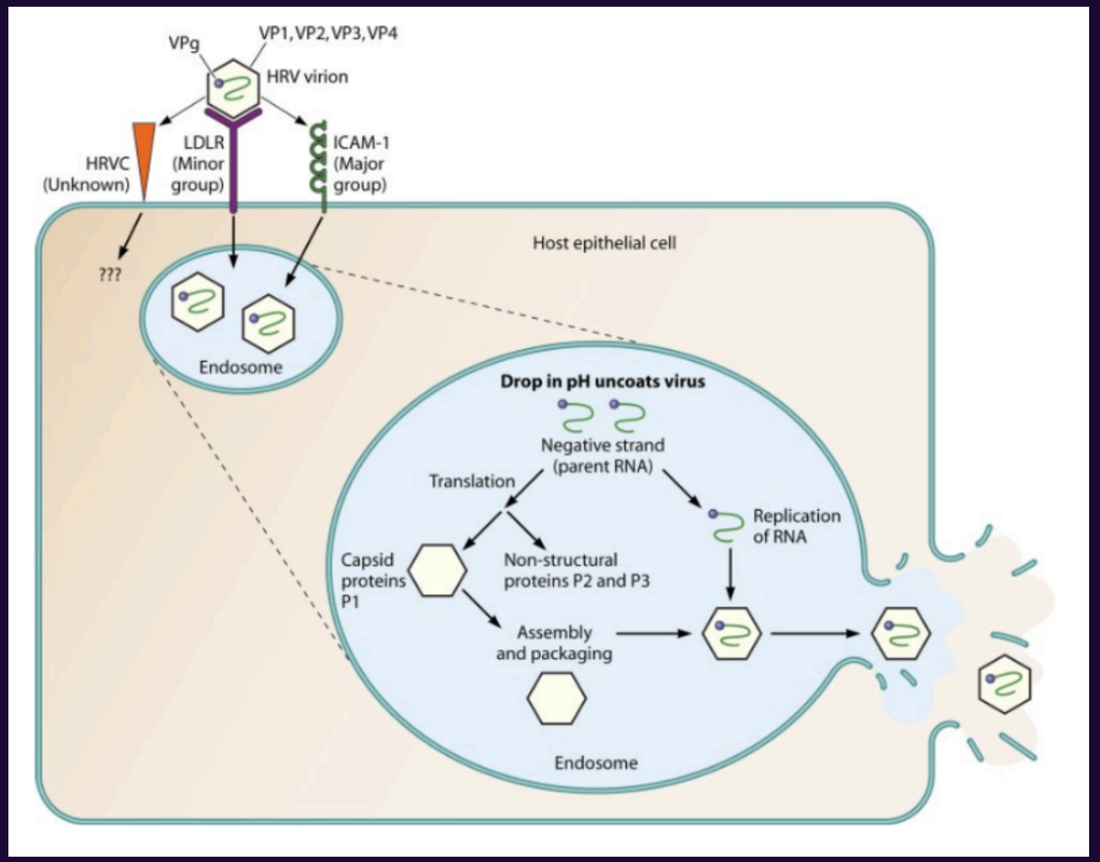
Serotypes: HRV-A, HRV-B, HRV-C

Rhinovirus 14

Viral replication

- HRV binds to specific cellular receptors (**ICAM-1** and **LDLR**)
- HRV enters the cell via endocytosis
- A drop in pH inside the endosome induces **uncoating** of the virus → release of the viral RNA into the cytoplasm
- The **positive strand RNA** acts as a messenger RNA

  Translated to produce capsid proteins and non-structural proteins
- The **negative strand RNA** is synthesized and used to replicate the viral RNA
- New RNA copies and capsid proteins assemble and form **new virions**
- The new virions are packed and **exit the cell**



Human Rhinoviruses- PMC

Capsid structure

Rhinovirus have a naked 30nm **icosahedral capsid** composed of 60 identical tightly packed protomers

Early in morphogenesis, immature protomers contain VP1, VP3 and VP0.

VP0 is later cleaved into VP2 and VP4 ⟶ Forming the **mature capsid**

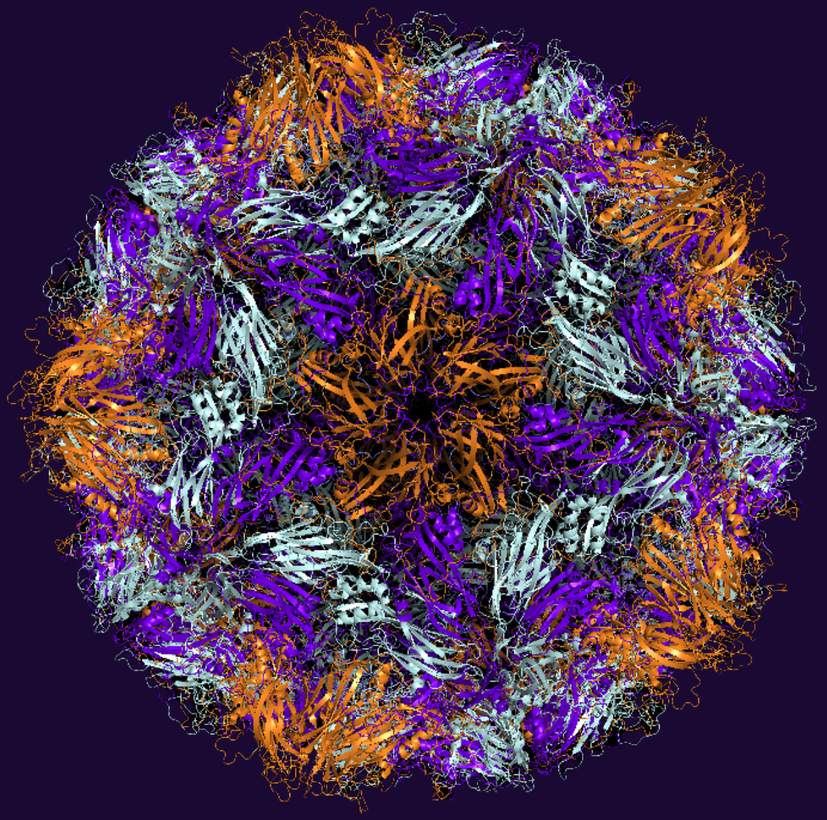
Virus assembly goes through a **dodecahedral pathway**, by the association of pentamers formed by five immature protomers leading to the icosahedral particle, defining 5-, 3-, and 2-fold symmetry axes.

Large proteins
(VP1, VP2, VP3)

Form the external and internal capsid surface

Small protein
(VP4)

Located inside the capsid only



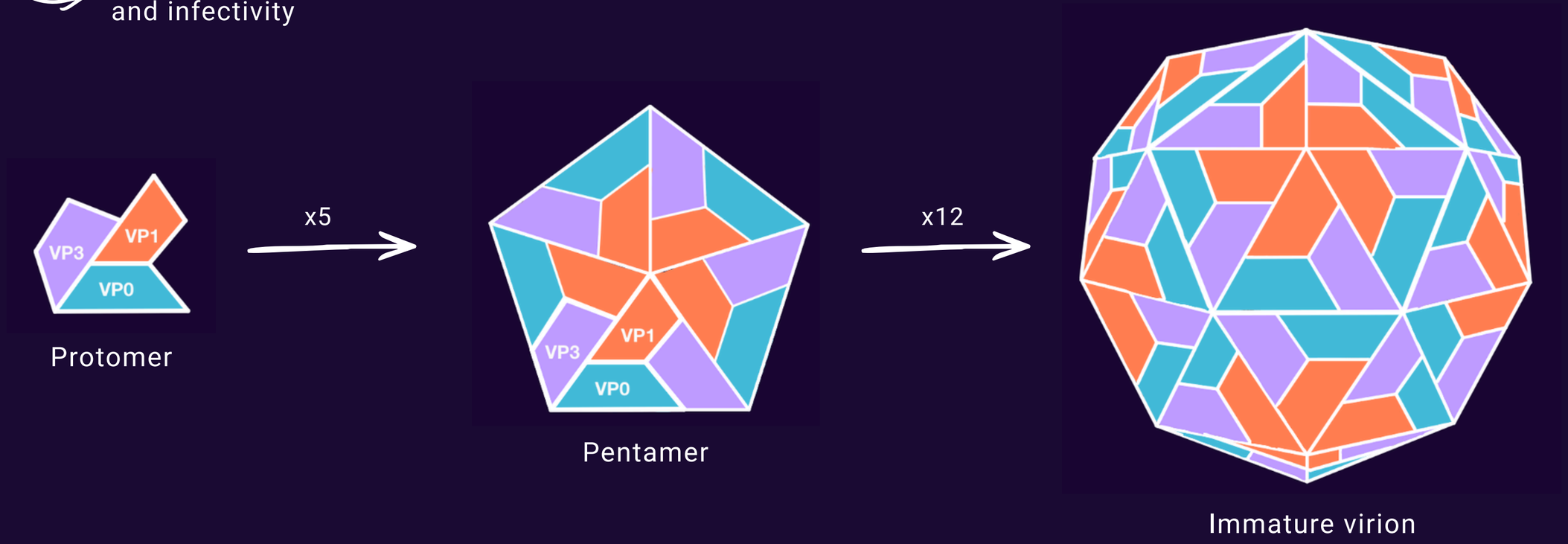
Chimera generated

Capsid structure

Capsid assembly

- The capsid assembles from **5 protomers** (VP0, VP1, and VP3) forming a **pentamer**
- **12 pentamers** come together, encapsulating the viral ssRNA to form an **immature virion**
- Then, VP0 undergoes autoproteolysis to generate **VP2** and **VP4** → Interacts with the RNA, stabilizes the capsid and prevents premature RNA release

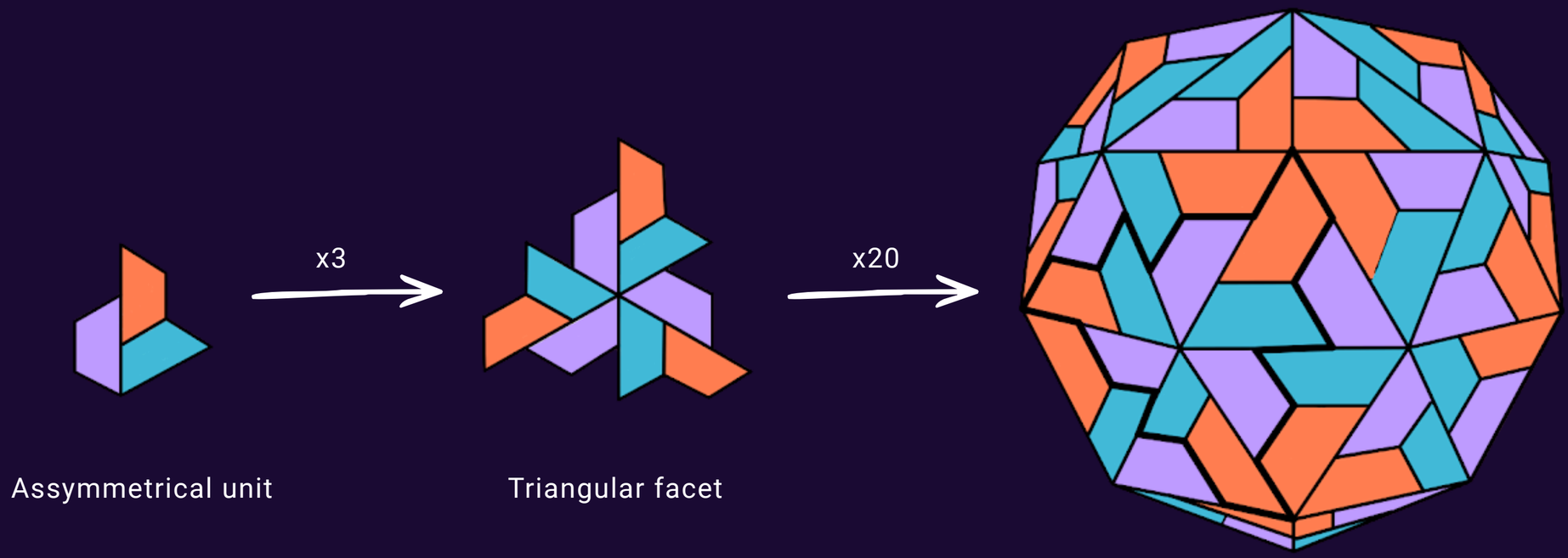
  ↳ This cleaveage is essential for the virion's stability and infectivity



Capsid structure

Pseudo T=3 organization

The capsid is made up of 60 asymmetric units, each consisting of three different proteins (T=3), totaling 180 proteins



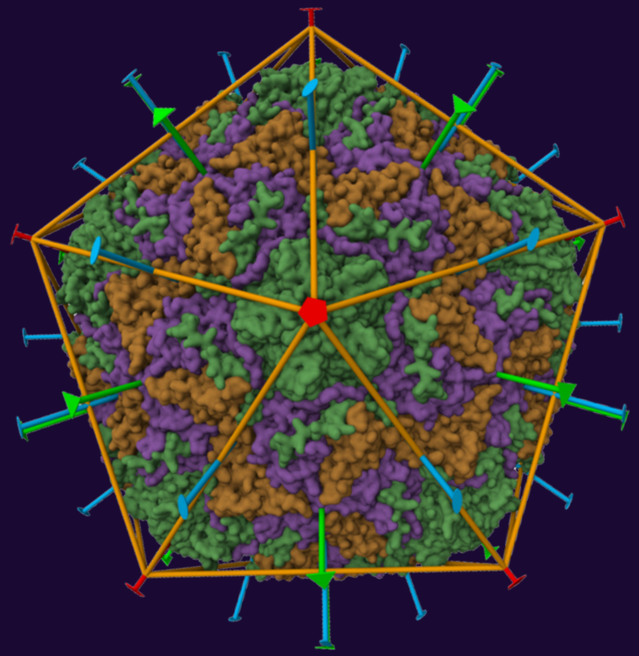
Due to the morphological similarity of the subunits, the structure is classified as **pseudo T=3**, rather than the regular T=3 symmetry described by Caspar and Klug

Capsid structure

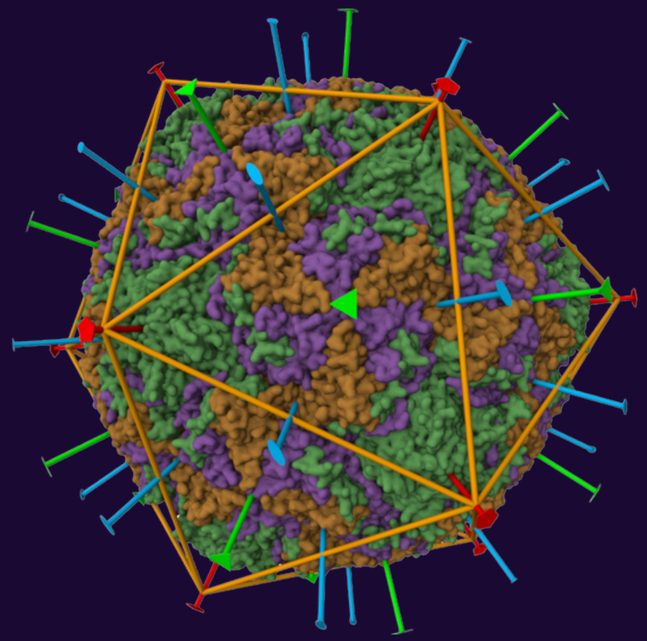
Icosahedral symmetry

The icosahedral structure of the rhinovirus capsid is defined by rotational symmetries, represented by these 3 axes:

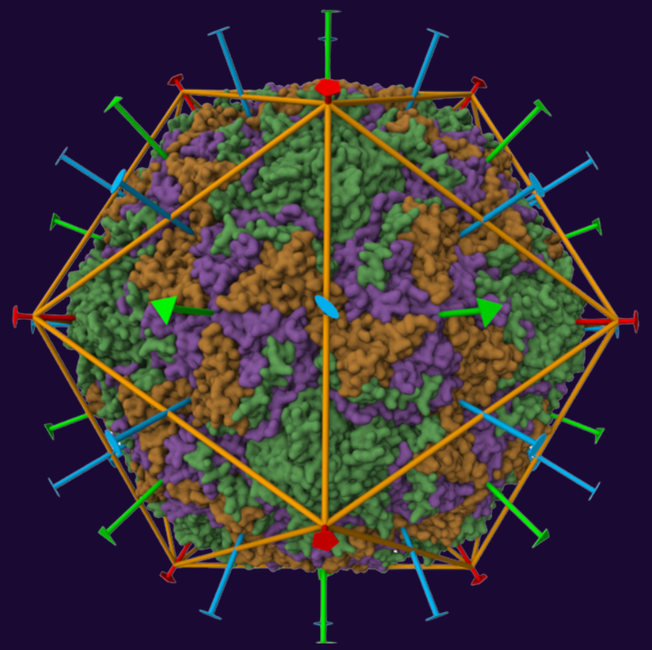
5-fold axis



3-fold axis



2-fold axis



VP1 VP0 VP3

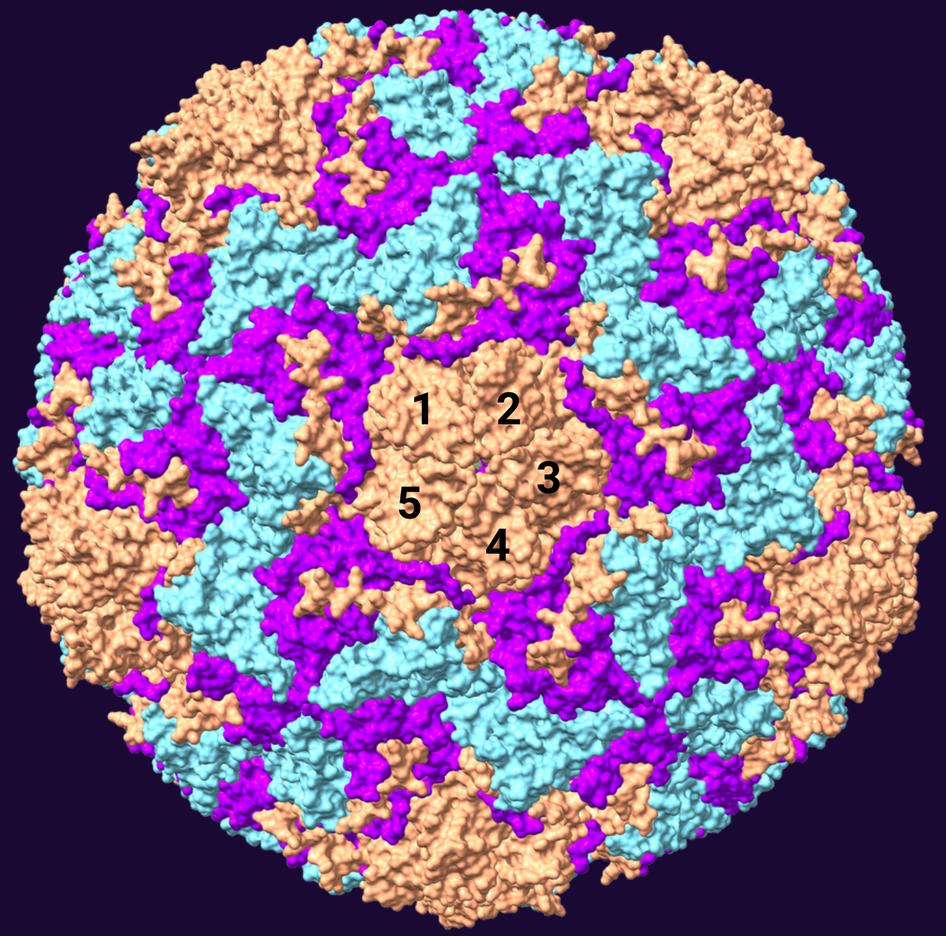
PDB obtained

Capsid structure

Icosahedral symmetry

Five copies of **VP1** assemble along the 5-fold axis

There is a total of 12 vertices with 5-fold axis



VP1 VP0 VP3

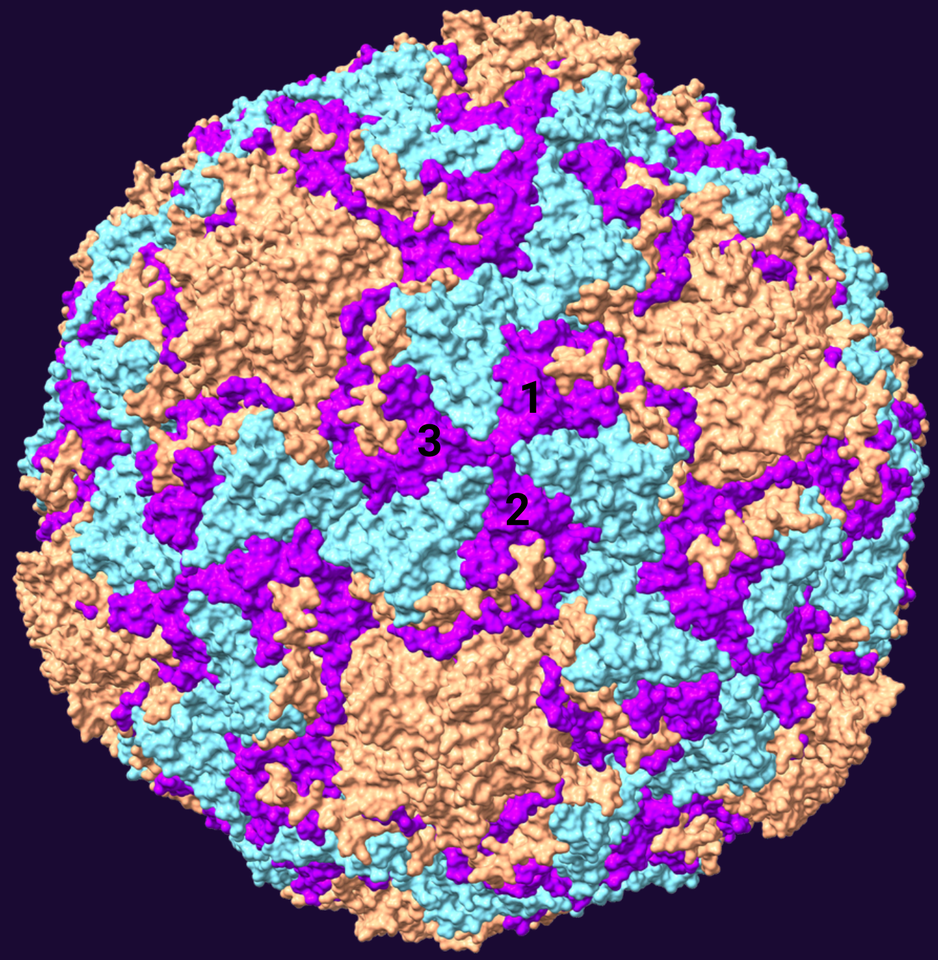
Chimera generated

Capsid structure

Icosahedral symmetry

Three alternating copies **VP3** assemble along the 3-fold axis

There is a total of 20 triangular faces with 3-fold axis



VP1 VP0 VP3

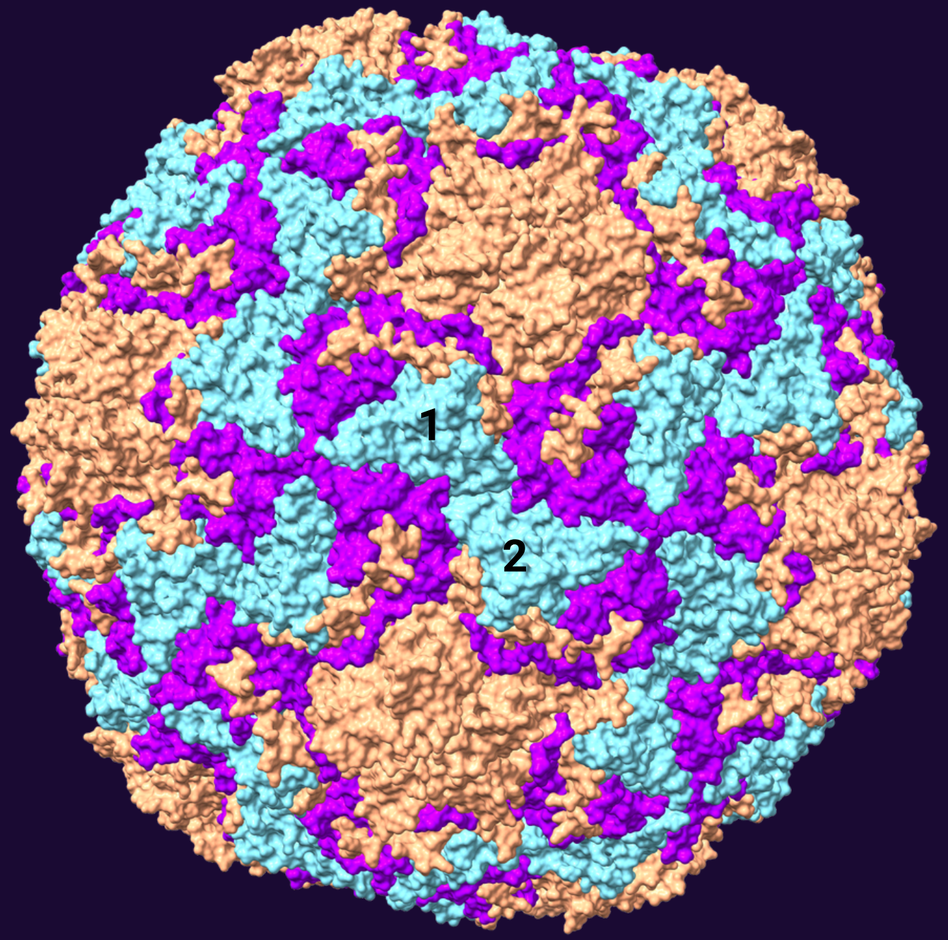
Chimera generated

Capsid structure

Icosahedral symmetry

Two copies of **VP0** interact with each other in the 2-fold axis assembly

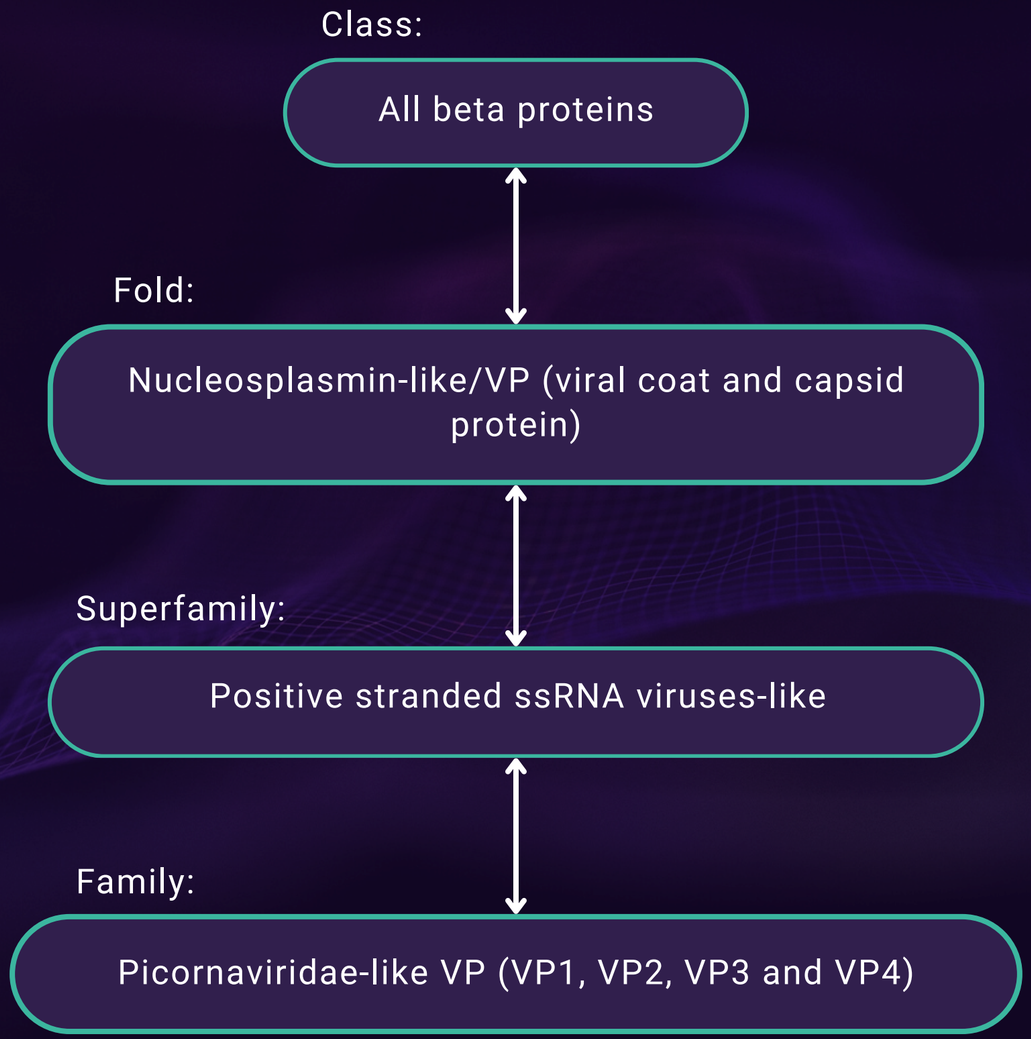
There is a total of 30 edges with 2-fold axis



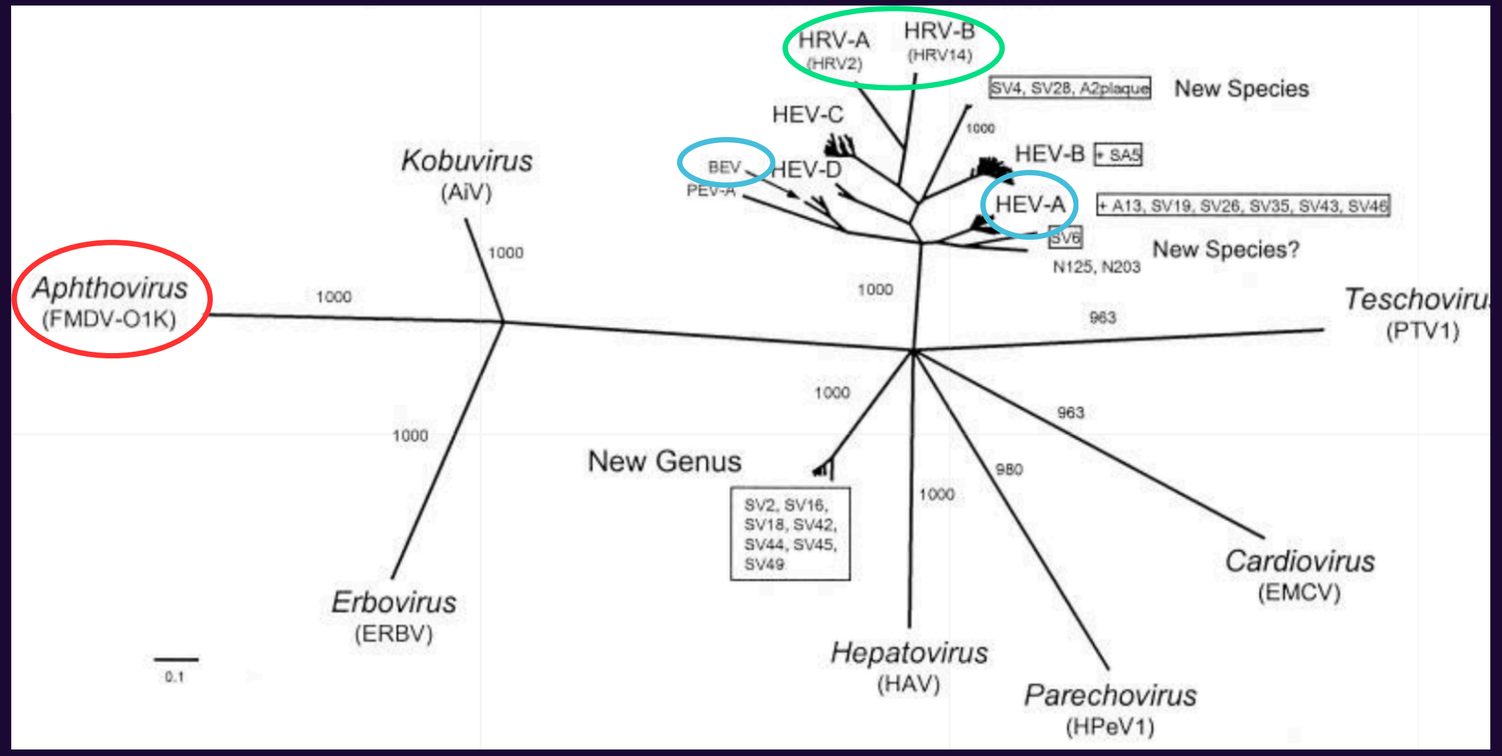
VP1 VP0 VP3

Chimera generated

SCOP classification



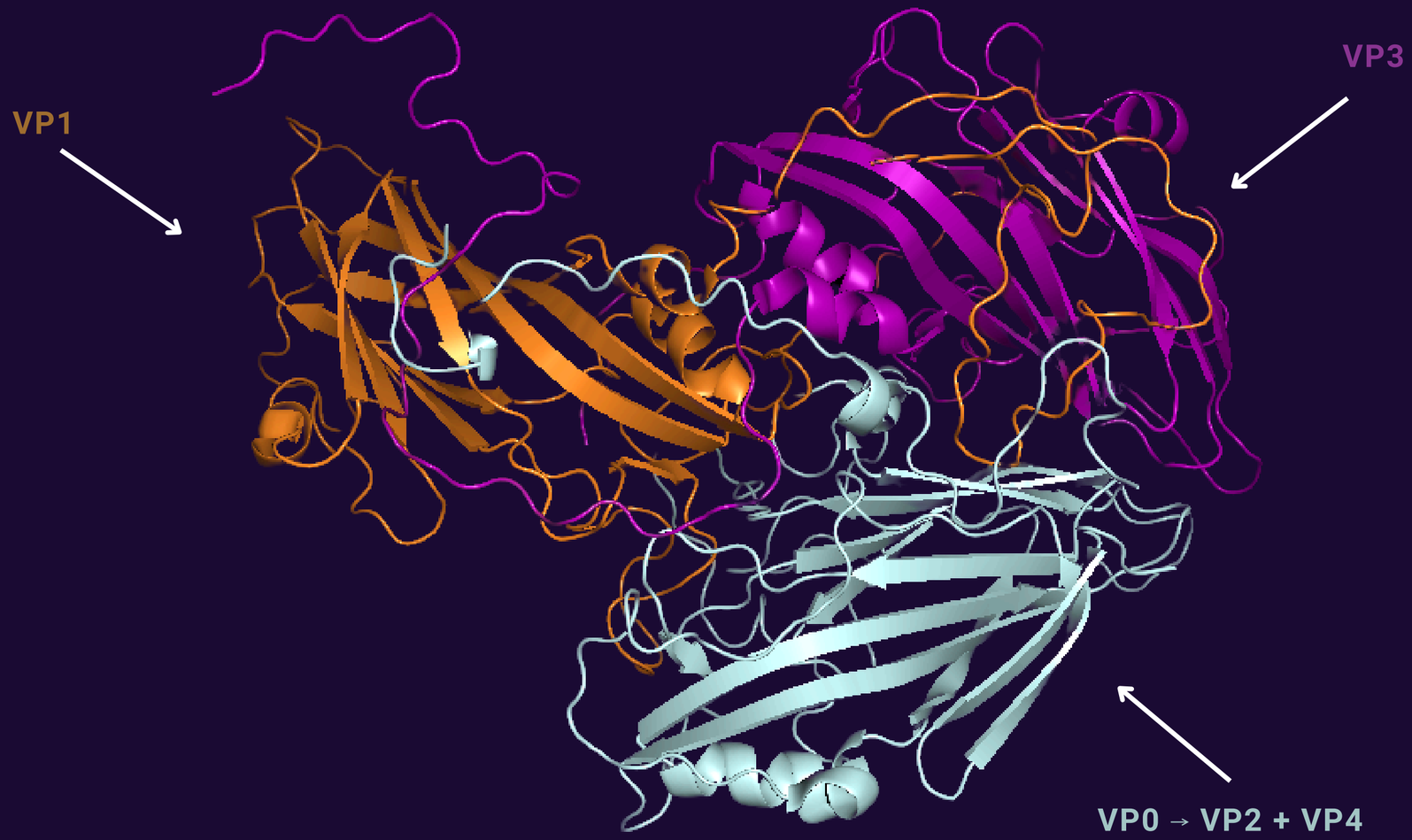
VPs Multiple Sequence Alignment



Molecular evolution of the human enteroviruses: correlation of serotype with VP1 sequence and application to picornavirus classification - PubMed

- 2 other Human Rhinoviruses: *Human rhinovirus B3* (1RHI), *Human Rhinovirus A16* (1ND2)
- 3 other Enteroviruses: *Human Poliovirus 1* (2PLV), *Echovirus 1* (1EV1), *Bovine Enterovirus VG-5-27* (1BEV)
- 1 other Picornavirus: *Foot and mouth virus* (1BBT)

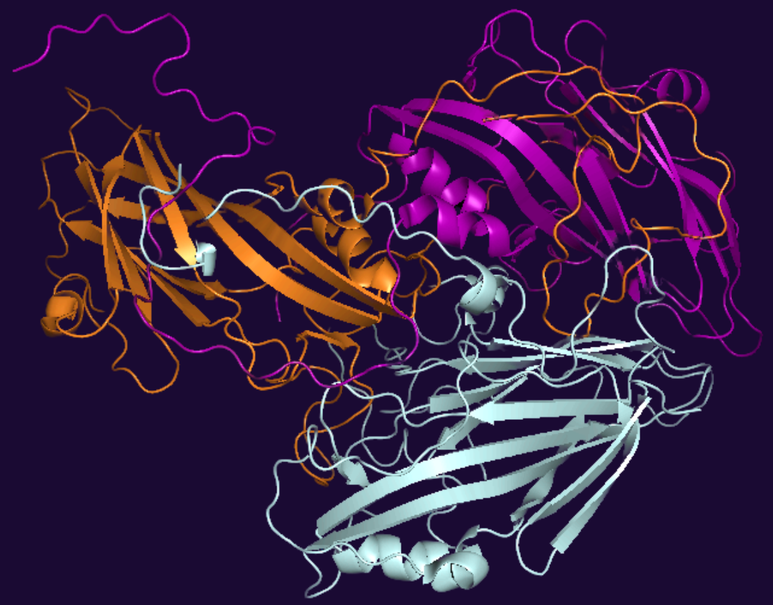
Capsid proteins - VP0, VP1 and VP3



Assymetrical unit

Pymol generated

Capsid proteins - VP0, VP1 and VP3

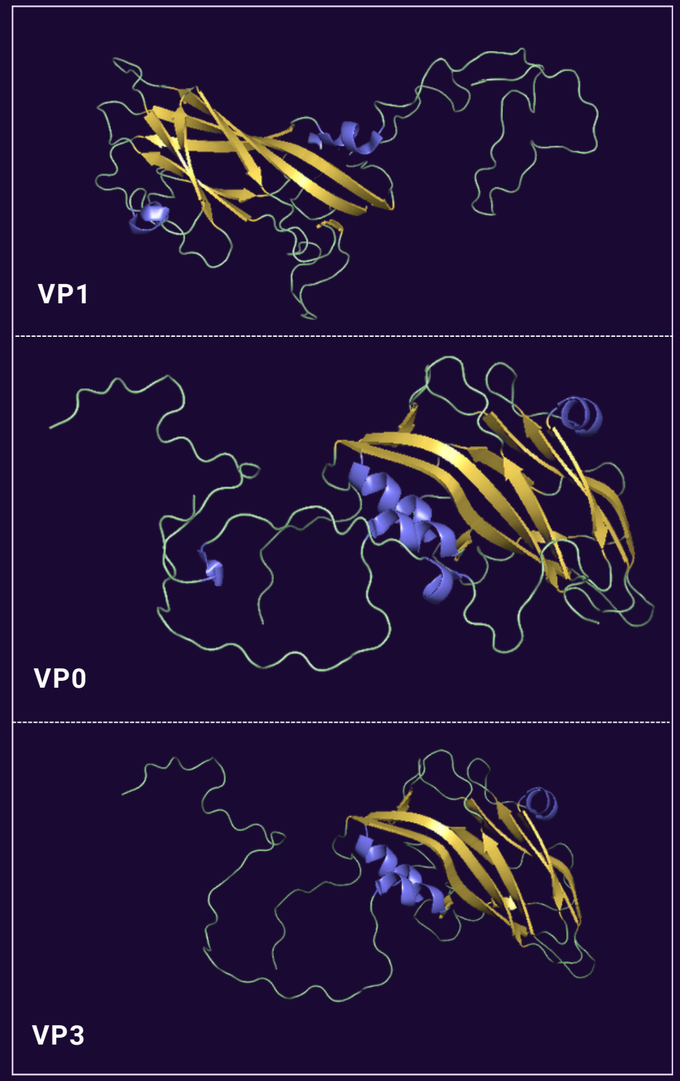


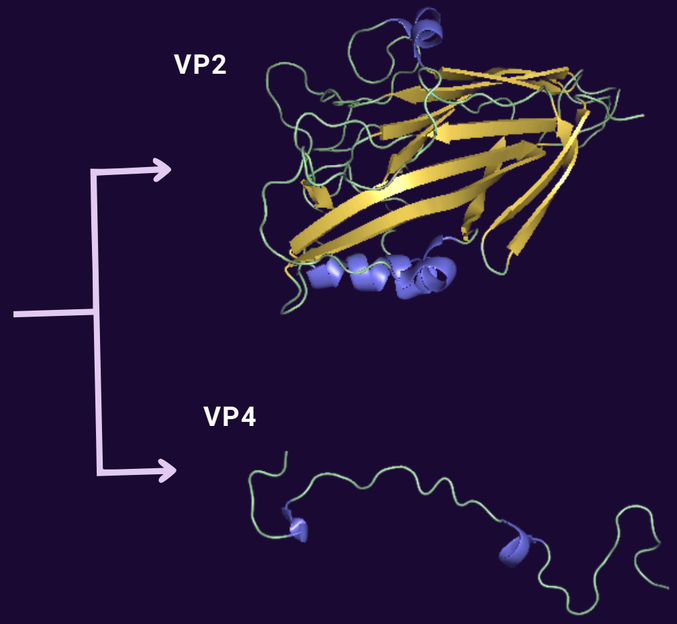
Assymetrical unit

VP1

VP0

VP3





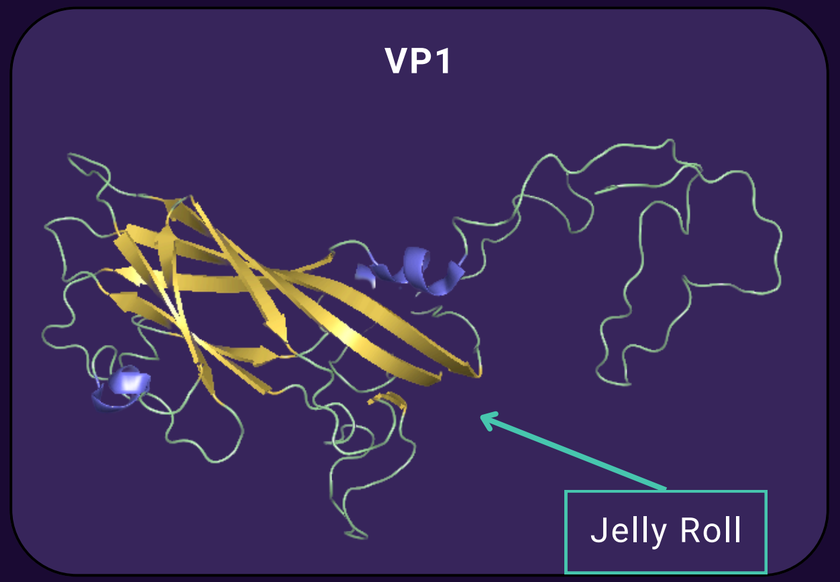
Anchors the ssRNA
to the capsid

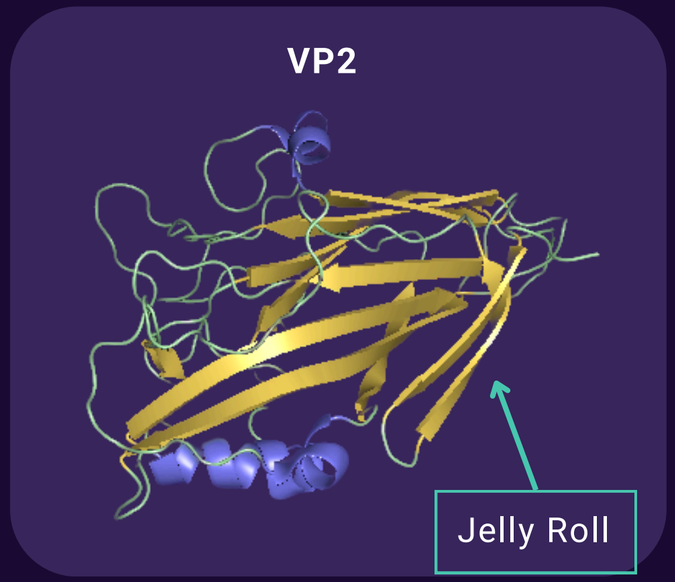
Pymol generated

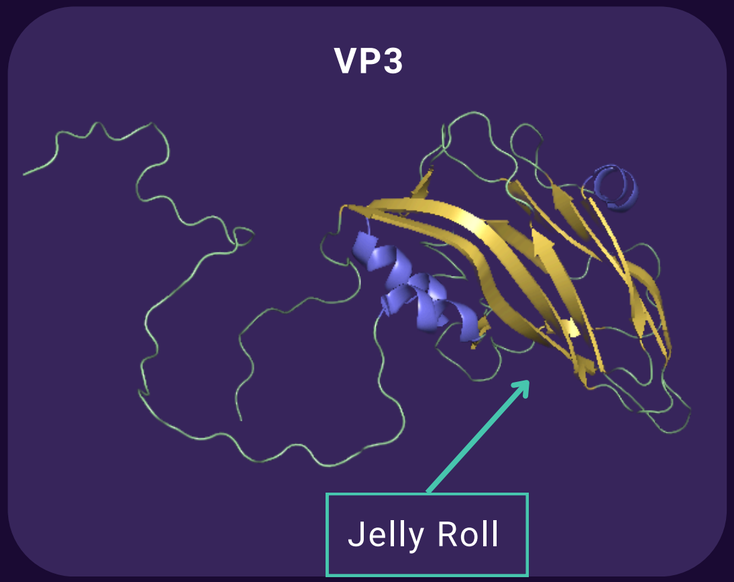
Capsid proteins - VP1, VP2 and VP3

Capsid proteins structure

The VP1, VP2, and VP3 proteins contribute to the virus's antigenic diversity by defining different serotypes







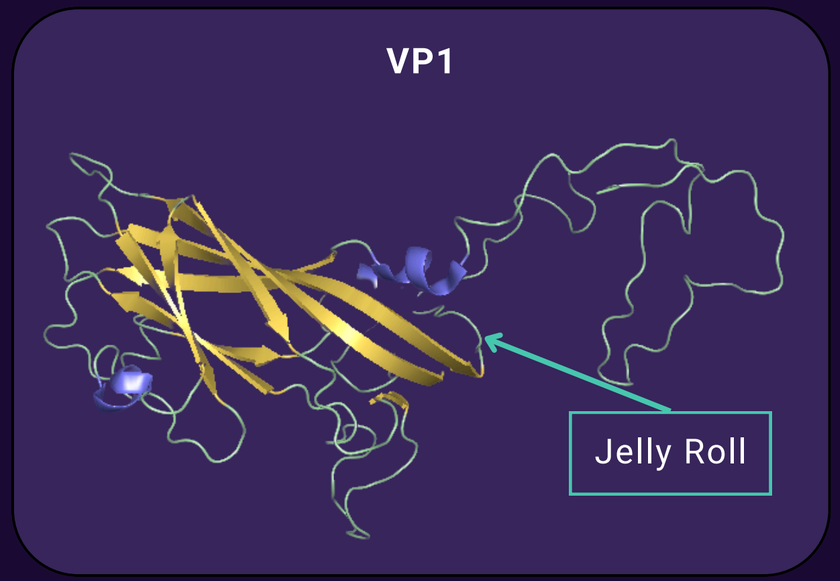
- The jelly roll fold consists of two β-sheets, each composed of 4 antiparallel β-strands, arranged in a twisted, roll-like structure.

Pymol generated

Capsid proteins - VP1, VP2 and VP3

Capsid proteins structure - VP1

VP1 participates in the uncoating process



- The **hydrophobic pocket** within the VP1 beta-barrel plays a key role in the uncoating process.
- Conformational changes, induced by receptor binding and/or acidification, result in:
  - The externalization of the **N-terminal segments** of VP1, as well as the release of VP4 and viral ssRNA through **transient openings** in the capsid.
- These transient openings occur at the interfaces between neighboring pentamers in the capsid, along the **2-fold axis**.

Some antiviral agents that inhibit the uncoating of picornaviruses bind to the hydrophobic pocket in the viral structure.

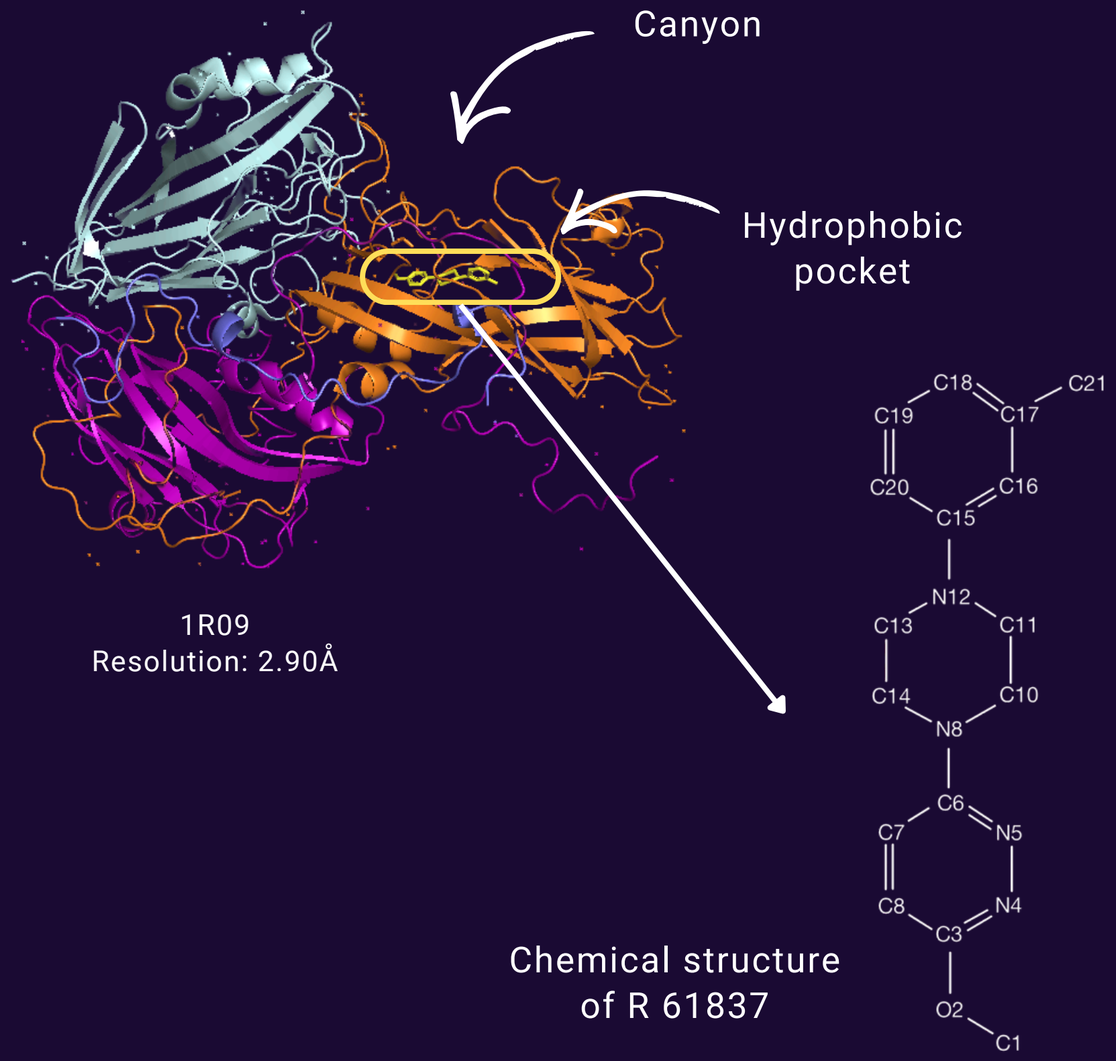
Pymol generated

Capsid proteins - VP1, VP2 and VP3

VP1 - Hydrophobic pocket

Antiviral Compound R 61837

- The antiviral compound R 61837 binds to the hydrophobic pocket beneath the floor of the canyon.
- By filling the pocket, antiviral compounds interfere with the virus's uncoating by making the protein coat less flexible.
- This binding induces conformational changes in the viral capsid around the pocket, including a significant movement of Met221 in VP1 and other residues in the FMDV loop.
- The FMDV loop is part of the surface of the canyon, where the ICAM-1 receptor binds.

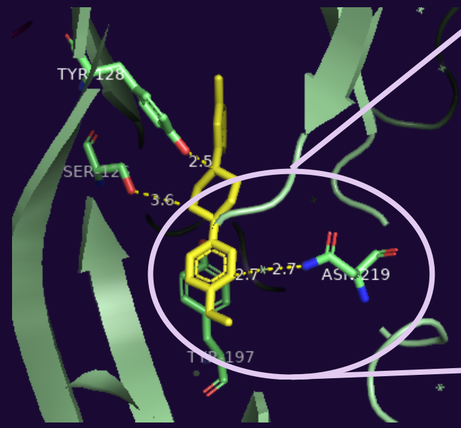


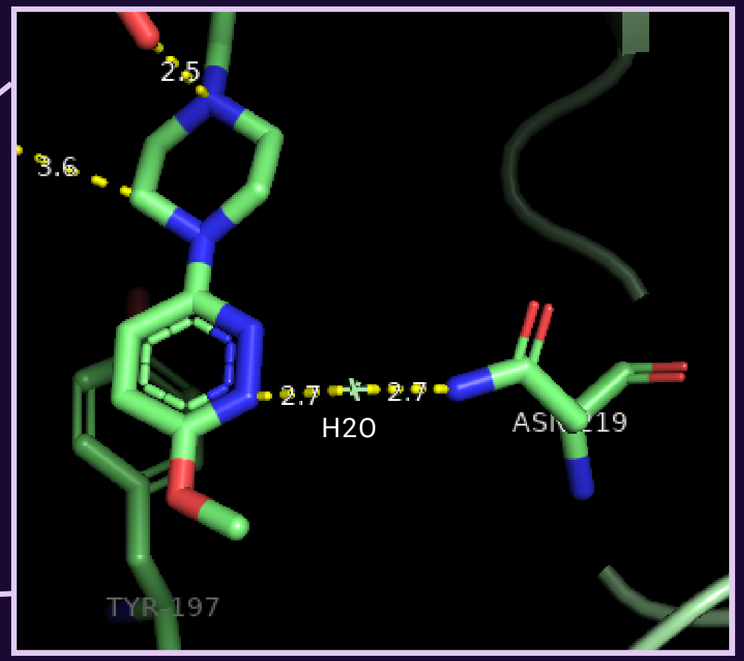
Pymol generated

Capsid proteins - VP1, VP2 and VP3

VP1 - Polar interactions in the hydrophobic pocket

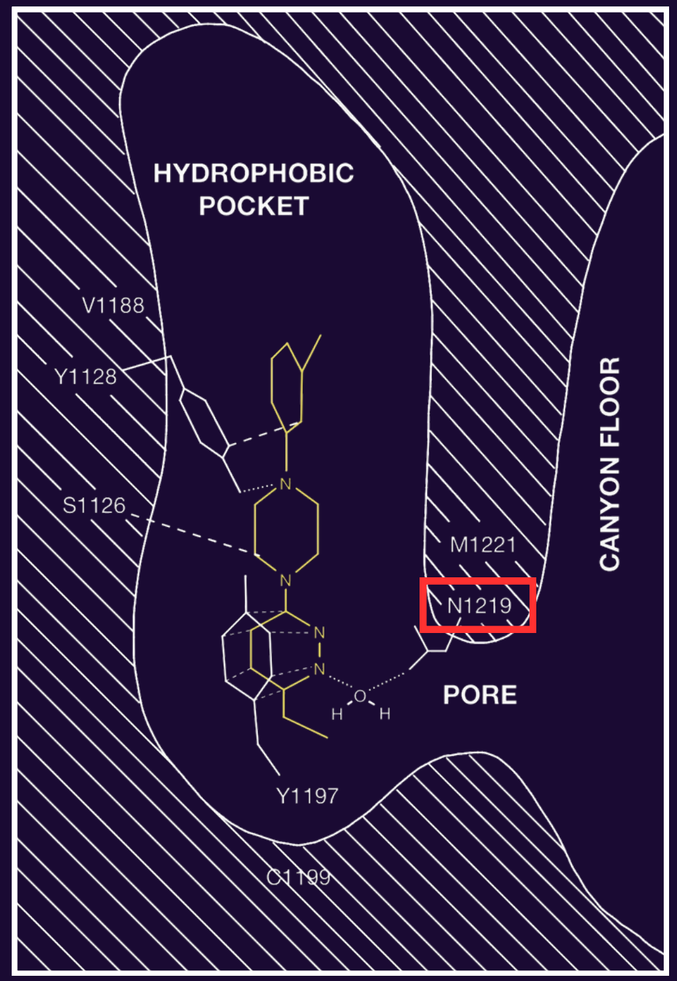
Antiviral Compound R 61837





Hidrogen bond:
N4 Pyridazine ring (R 61837) - NH2 Asn 219 (VP1)
H20 mediated

The distance of both interactions is 2.7 Å



Pymol generated

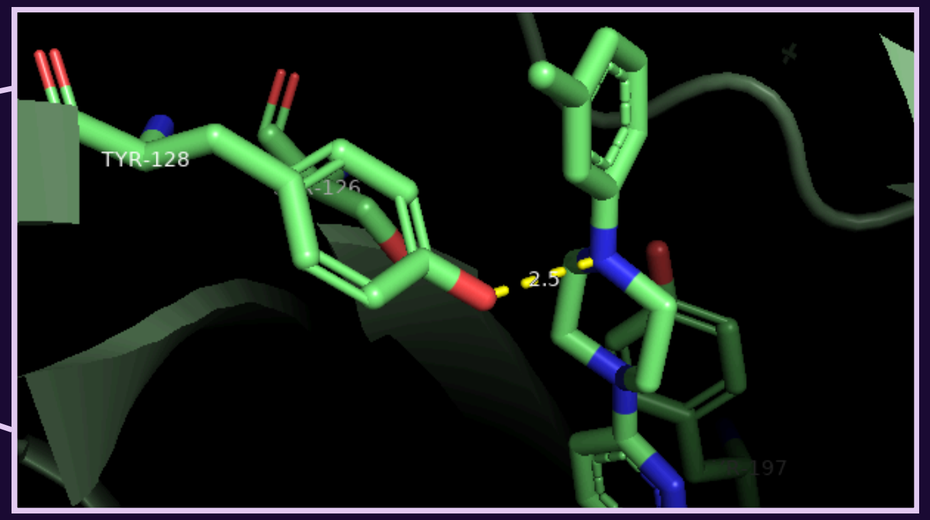
Capsid proteins - VP1, VP2 and VP3

VP1 - Polar interactions in the hydrophobic pocket

Antiviral Compound R 61837

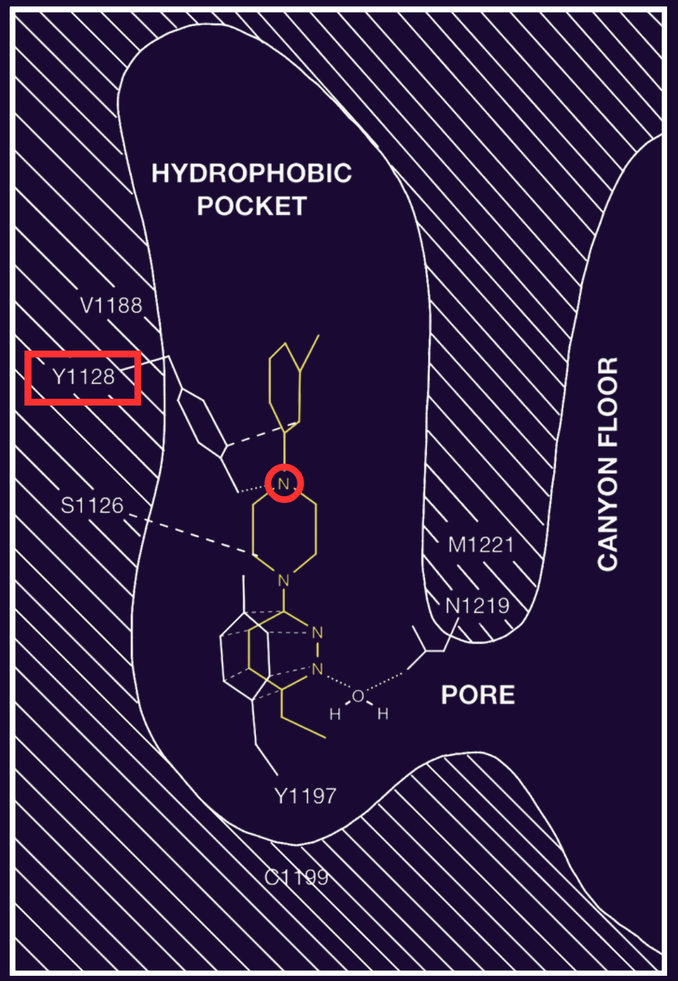


R 61837



Hidrogen bond:
OH Tyr 128 (VP1) - N12 Piperazine ring (R 61837)

The distance of this interaction is 2.5 Å



Pymol generated

Capsid proteins - VP1, VP2 and VP3

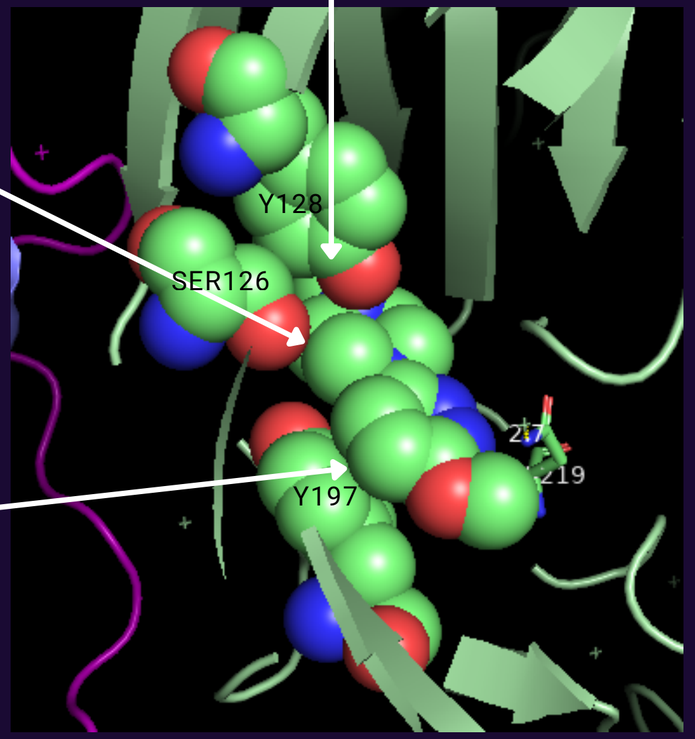
VP1 - Hydrophobic interactions in the hydrophobic pocket

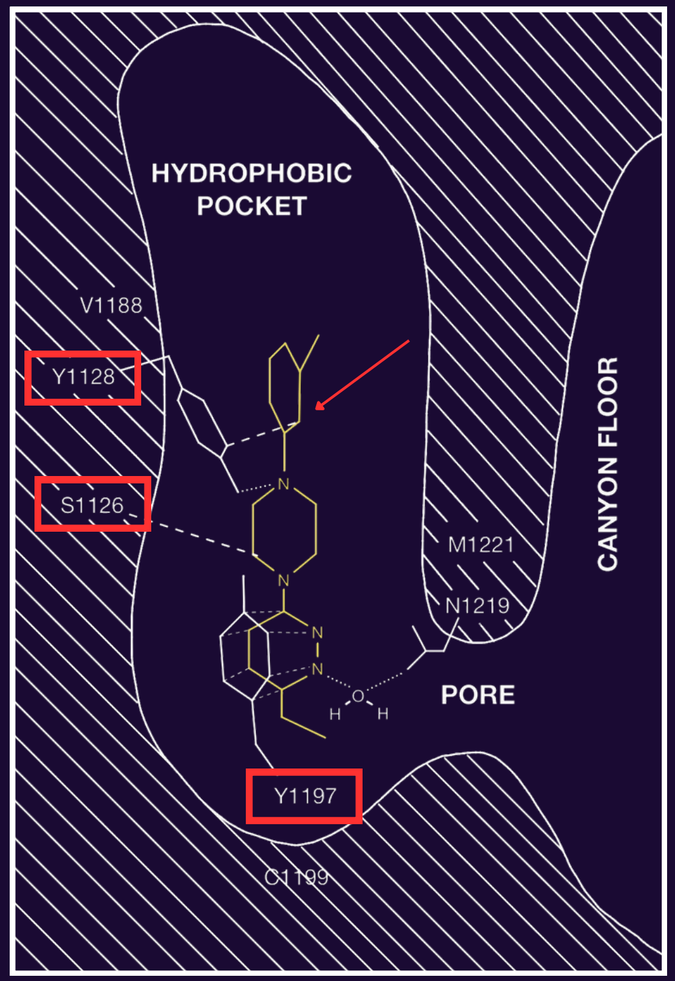
Antiviral Compound R 61837

vdW force
Cεl TYR128 (VP1) - C16 Phenyl (R61837)
3.2 Ä

vdW force
Oy Ser 126 (VP1) - C14 Piperazine (R61837)
3.6 Ä

π-π interaction
TYR197 (VP1) - Pirydazine (R61837)
4.2 Ä

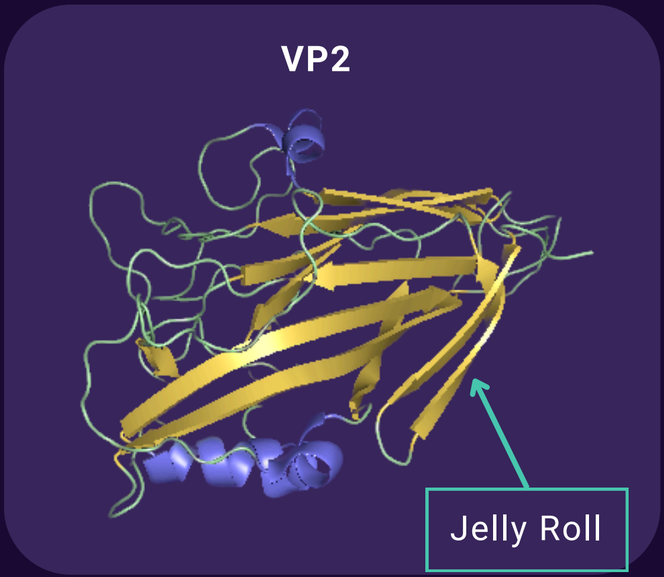




Pymol generated

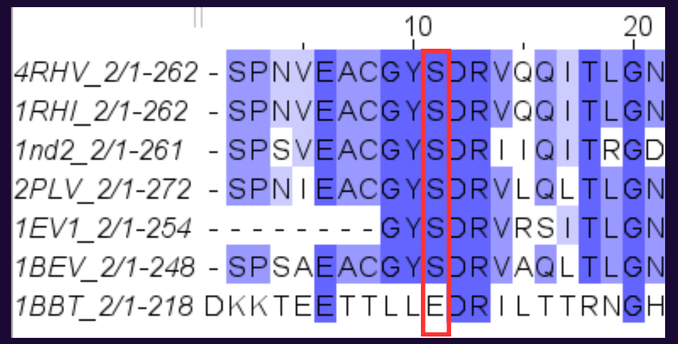
Capsid proteins - VP1, VP2 and VP3

Capsid proteins structure - VP2

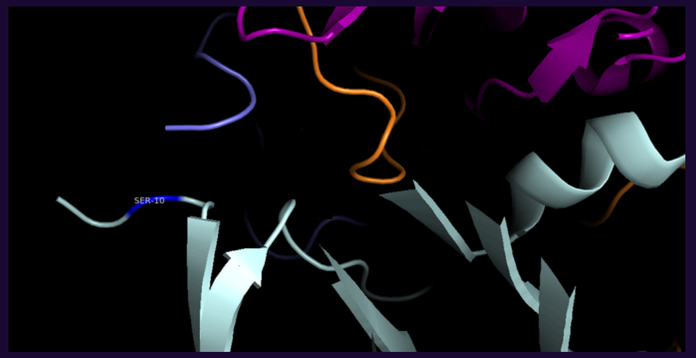


- The separation of VP0 into VP2 and VP4 takes place during virion maturation.
- Serine 10 in VP2 is located next to the carboxyl terminus of VP4. The conservation of this residue across different picornaviruses suggests the involvement of an autoproteolytic mechanism.
- It is possible that an RNA base acts as a catalyst, converting the serine hydroxyl group into a more nucleophilic alkoxide form.

4RHV: *Human Rhinovirus B14*
1RHI: *Human Rhinovirus B3*
1ND2: *Human Rhinovirus A16*
2PLV: *Human Poliovirus 1*
1EV1: *Echovirus 1*
1BEV: *Bovine Rhinovirus*
1BBT: *Foot and Mouth virus VG-5-27*



HMM alignment

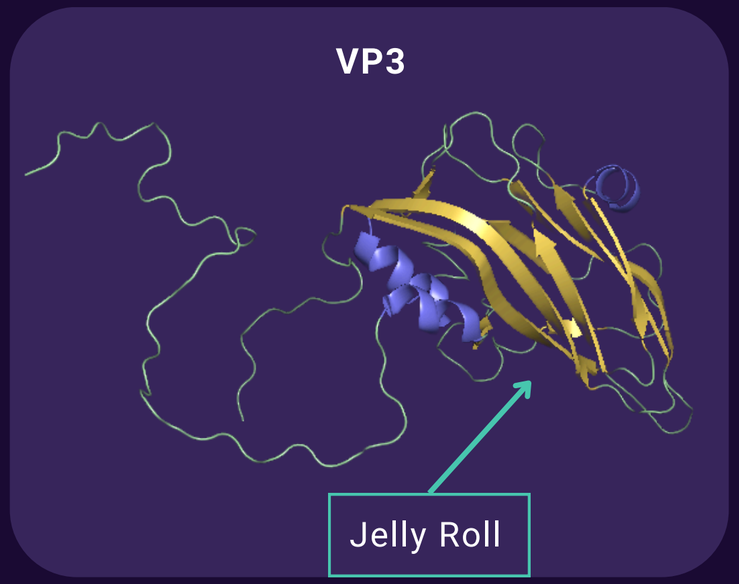


Serine 10, located near the carboxyl terminus of VP4

Pymol generated

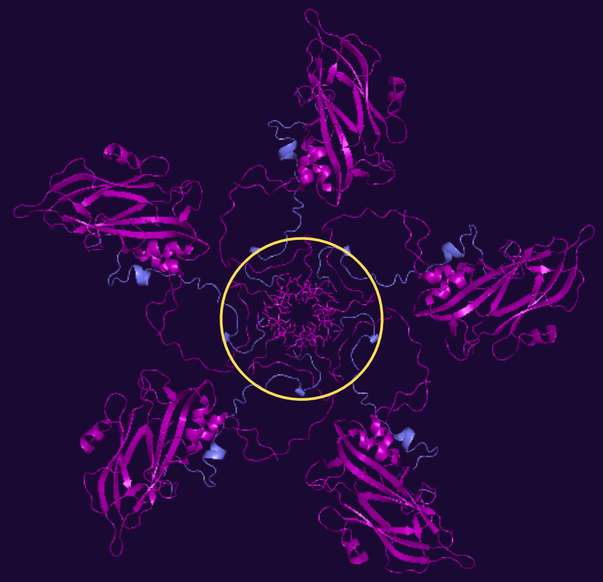
Capsid proteins - VP1, VP2 and VP3

Capsid proteins structure - VP3



VP3 is crucial in the assembly of pentamers, as five VP3 molecules come together to form a structure known as the β-cylinder.

The β-cylinder is formed by the N-terminal ends of five VP3 molecules within a pentamer, which are surrounded by the N-terminal ends of VP4 molecules.



VP3

VP4

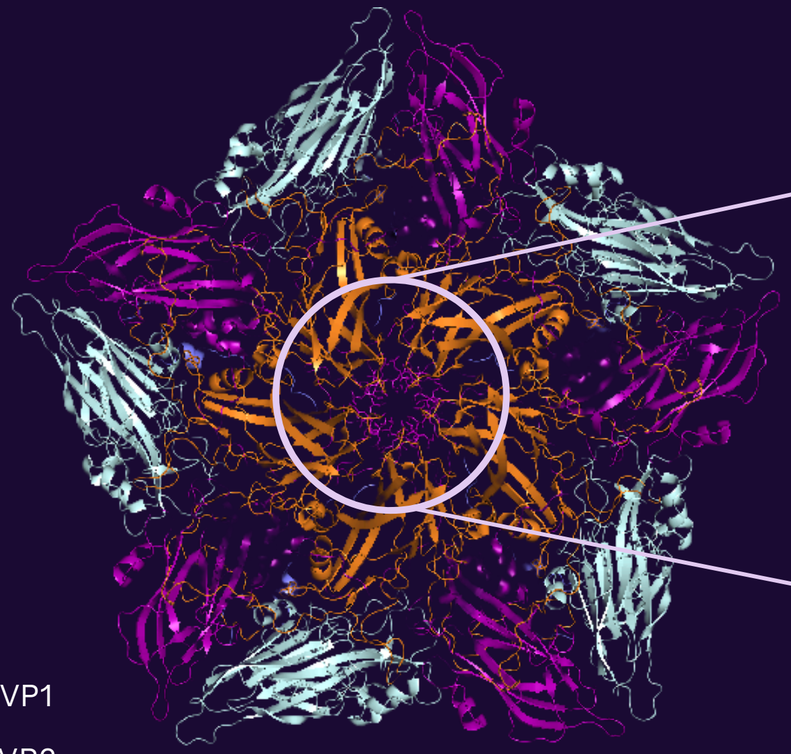
The β-cylinder plays a key role in pentamer formation during:

- Capsid assembly
- Pentamer stabilization
- Pentamer breakdown during disassembly

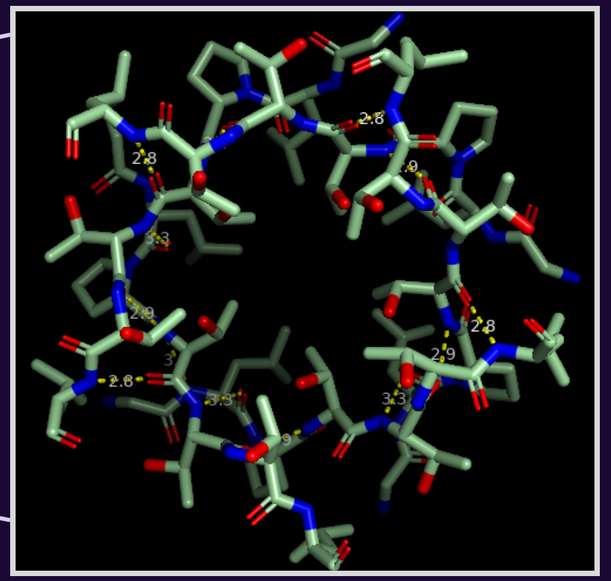
Pymol generated

Capsid proteins - VP1, VP2 and VP3

VP3 - β-cylinder



VP1
VP2
VP3
VP4



These five VP3 molecules arrange their N-terminal ends to create a parallel β-sheet with a cylindrical structure.

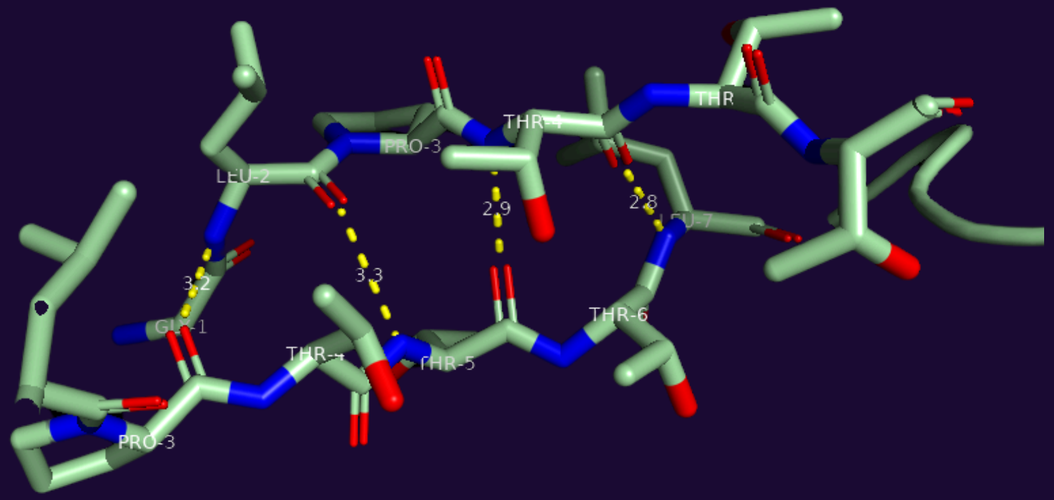
How do they interact?

Pymol generated

Capsid proteins - VP1, VP2 and VP3

VP3 - β-cylinder

Each β-strand within the cylinder forms eight hydrogen bonds, with four bonds connecting to each adjacent strand, stabilizing the overall structure.



N-terminal sequence:

G L P T T T L

G L P T T T L — 2.8 Å — G L P T T T L

G L P T T T L — 2.9 Å — G L P T T T L

G L P T T T L — 3.3 Å — G L P T T T L

G L P T T T L — 3.2 Å — G L P T T T L

Pymol generated

VP1-3 sequence comparison

CLUSTAL ALIGNMENT



HMM ALIGNMENT



- Low sequence identity
- Inconsistent alignment of secondary structure elements
- Conserved residues scattered, no extended conserved blocks
- **Homology not clearly detectable at sequence level**

VP1-3 sequence comparison

CLUSTAL ALIGNMENT

```
CLUSTAL 2.1 multiple sequence alignment


VP1             -------GLG-DELEEVIVEKTKQTVASISSGPKHTQKVPILTANETGATMPVLPSDSIE
VP3             -------GLPTTTLPGSGQFLTTDDRQSPSALPNYEPTPRIHIPGKVHNLLEIIQVDTLI
VP2             SPNVEACGYSDRVQQITLGNSTITTQEAANAVVCYAEWPEYLPDVDASDVNKTSKPDTSV
                       *             *     : .:   :          ..         *:

VP1             TRTTYMHFNGSETDVECFLGRAACVHVTEIQNKDATGIDNHREAKLFNDWKINLSSLVQL
VP3             P----MNNTHTKDEVNSYLIPLN----ANRQNEQVFGTNLFIGDGVF--------KTTLL
VP2             CRFYTLDSKTWTTGSKGWCWKLP----DALKDMGVFGQNMFFHSLGR--------SGYTV
                     :. .      : :            ::  . * : .              .   :

VP1             RKKLELFTYVRFDSEYTILATASQPDSANYSSNLVVQAMYVPPGAPNPKEWDDYTWQSAS
VP3             GEIVQYYTHWSGSLRFSLMYTGPALSSAK------LILAYTPPGARGPQDRRE----AML
VP2             HVQCNATKFHSGCLLVVVIPEHQLASHEG--GNVSVKYTFTHPGERGIDLSSANEVGGPV
                    :  ..        ::      .         :   :. **  . .        .

VP1             NPSVFFKVGDTSRFSVPYVGLASAYNCFYDGYSHDDAETQYGITVLNHMGSMAFRIVNEH
VP3             GTHVVWDIGLQSTIVMTIPWTSGVQ------FRYTDPDTYTSAGFLSCWYQTSLILPPET
VP2             KDVIYNMNGTLLGNLLIFPHQFINLRTNNTATIVIPYINSVPIDSMTRHNNVSLMVIPIA
                   :    *      :                          .      :.   . :: :

VP1             DEHKTLVKIRVYHRAKHVEAWIPRAPRALPYTSIGRTNYPKNTEPVIKKRKGDIKSY
VP3             TG------------QVYLLSFISACP-DFKLRLMKDTQTISQTVALTE---------
VP2             PL-------------TVPTGATPSLPITVTIAPMCTEFSGIRSKSIVPQ--------
                                    .  .  *  .    :       .: .:
```

HMM ALIGNMENT

```
CLUSTAL 2.1 multiple sequence alignment


VP1             ---------------------GLG-DELEEVIVEKTKQTVASISSGPKHTQKVP-ILTAN
VP3             ---------------------GLPTTTLPGSGQFLTTDDRQSPSALPNY-EPTPRIHIPG
VP2             -----------------------SPNVEACGYSDRVQQITLGNSTITTQEAANAVVCYA
                                        :        :     .:  .  : .  :

VP1             ETGATMPVLP-SDSIETRTTYMHFN-GSETDVECFLGRAACV--HVTEIQNKDAT---GI
VP3             KVHNLLEIIQ----VDTLIPMNNT--HTKDEVNSYLIPLNAN------RQNEQVF---GT
VP2             EWPEYLPDVDASDVNKTSKPDTSVCRFYTLDSKTWTTGSKGW-----CWKLPDAL--KDM
                :    :  :      .*  .          : : :             :  :.    .

VP1             DNHREAKLFNDWKINL----SSLVQLRKKLELFT------YVRFDSEYTILATASQPDSA
VP3             NLFIGDGVF---KTTL---------LGEIVQYYT------HWSGSLRFSLMYTG------
VP2             GVFGQNMFFHSLGRSG---------YTVHVQCNA------TKFHSGCLLVVVIPEHQLAS
                . .    .*     .             ::  :          .    ::

VP1             NYSSNLVVQAMYVPPGAP--------------NPKEW----------------------
VP3             PALSSAKLILAYTPPGAR--------------GPQDR----------------------
VP2             HEGGNVSVKYTFTHPGER--------------GIDLS----------------------
                   ..  :   :. **                . .

VP1             DDYTWQSASNPSVFFKVG--DTSRFSVPYVGLASAYNCFYDGYSHDDAETQYGITVLN-H
VP3             ----REAMLGTHVVWDIGLQSTIVMTIPWTSGVQFR------YTDPDT-----YTSA---
VP2             -SANEVGGPVKDVIYNMNGTLLGNLLIFPHQFINLR------TNNTATIVIPYINSVP--
                      .     *.:.:.      : :      .         ..  :      .

VP1             MGSMAFRIVNEHDEHKTLVKIRVYHRAKHVEAWIPRAPRALPYTSIGRTNYPKNTEPVIK
VP3             -GFLSCWYQ------TSLILPPETTGQVYLLSFISACP-DFKLRLMKDTQTISQTVALTE
VP2             IDSMTRHN-----NVSLMVI-PIAPLT-VPTGATPSLPITVTIAPMCTEFSGIRSKSIVP
                 . ::          . ::           .  .  * .    :      .: .:

VP1             KRKGDIKSY----------------------------
VP3             -------------------------------------
VP2             Q-------------------------------------
```

SEQUENCE DISSIMILARITY ≠ STRUCTURE DISSIMILARITY

VP1-3 structural comparison

STAMP

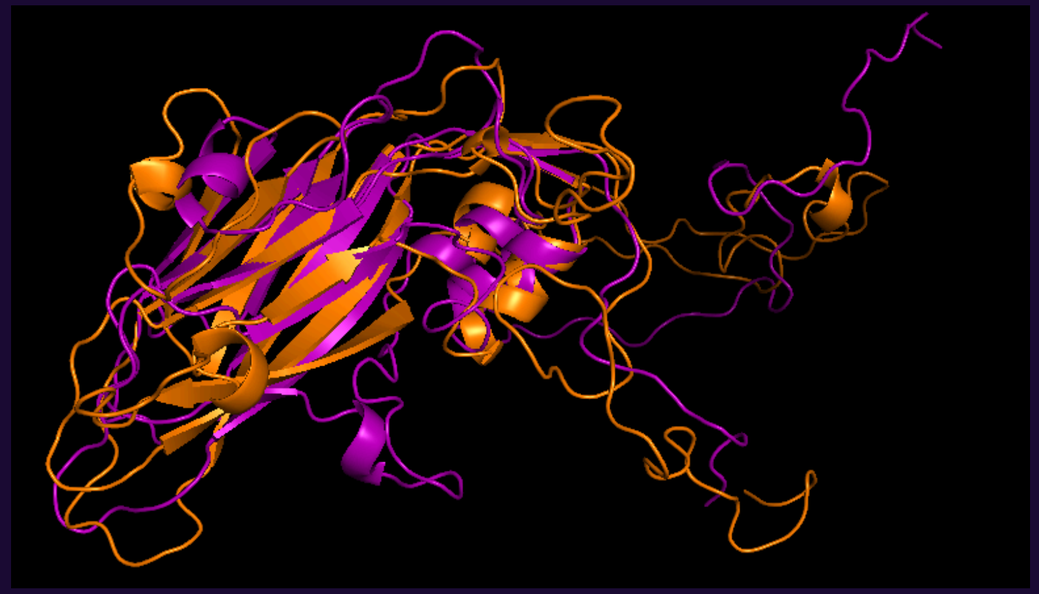
```
STAMP Structural Alignment of Multiple Proteins

Version 4.4 (May 2010)
 by Robert B. Russell & Geoffrey J. Barton
 Please cite PROTEINS, v14, 309-323, 1992

Running roughfit.

    Sc = STAMP score, RMS = RMS deviation, Align = alignment length
    Len1, Len2 = length of domain, Nfit = residues fitted
    Secs = no. equivalent sec. strucs. Eq = no. equivalent residues
    %I = seq. identity, %S = sec. str. identity
    P(m)  = P value (p=1/10) calculated after Murzin (1993), JMB, 230, 689-694
            (NC = P value not calculated - potential FP overflow)

     No.  Domain1         Domain2         Sc     RMS    Len1 Len2  Align NFit Eq. Secs.   %I   %S   P(m)
Pair   1  VP1             VP2             2.17   2.39    273  255   257  110 103    0  14.56 100.00 3.84e-02
Pair   2  VP1             VP3             3.19   2.29    273  236   288  128 113    0  10.62 100.00 1.00e+00
Pair   3  VP2             VP3             0.28   1.87    255  236   354    8   3    0   0.00 100.00 1.00e+00  LOW SCORE
Reading in matrix file capsid.mat...
Doing cluster analysis...
Cluster:  1 (     VP1  &      VP3 ) Sc  3.19 RMS   2.29 Len 288 nfit 128
 See file capsid.1 for the alignment and transformations
Cluster:  2 (     VP2  &      VP1      VP3 ) Sc  4.66 RMS   2.50 Len 279 nfit 140
 See file capsid.2 for the alignment and transformations
```



VP1-VP3 superposition.

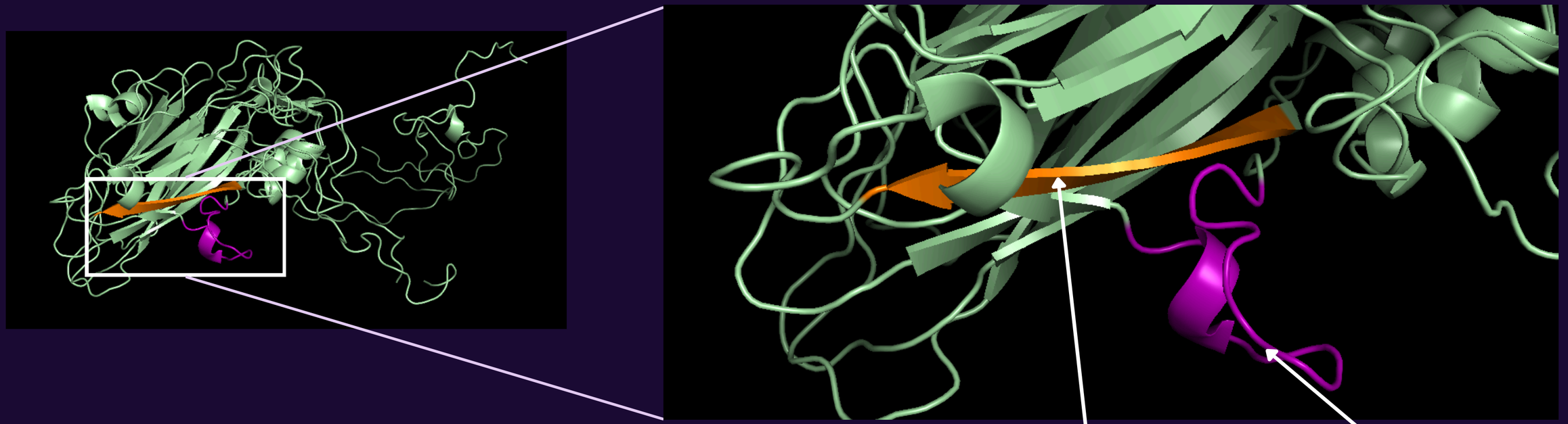
- VP1 and VP3: Stable cluster with RMSD = 2.39 → Significant structural similarity.
- VP2 decreases alignment quality → Increased RMSD and low score in the VP2-VP3 pair.
- VP2 is not enough similar for reliable superposition (possible variability in loops and beta structures).
- More complex analysis could focus on the structural core for better superposition of all three proteins.

VP1

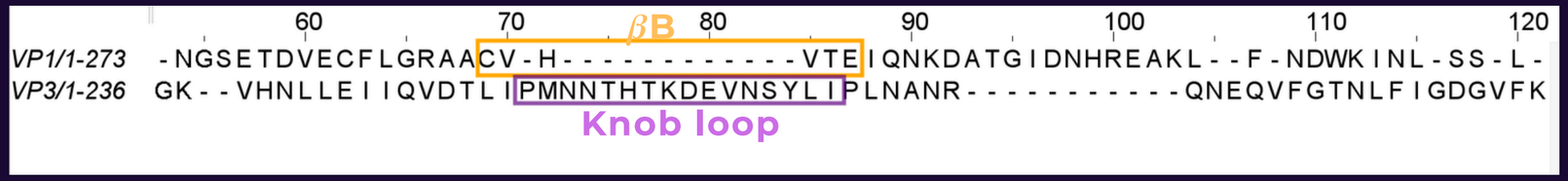
VP3

VP1-VP3 structural comparison

Variable regions

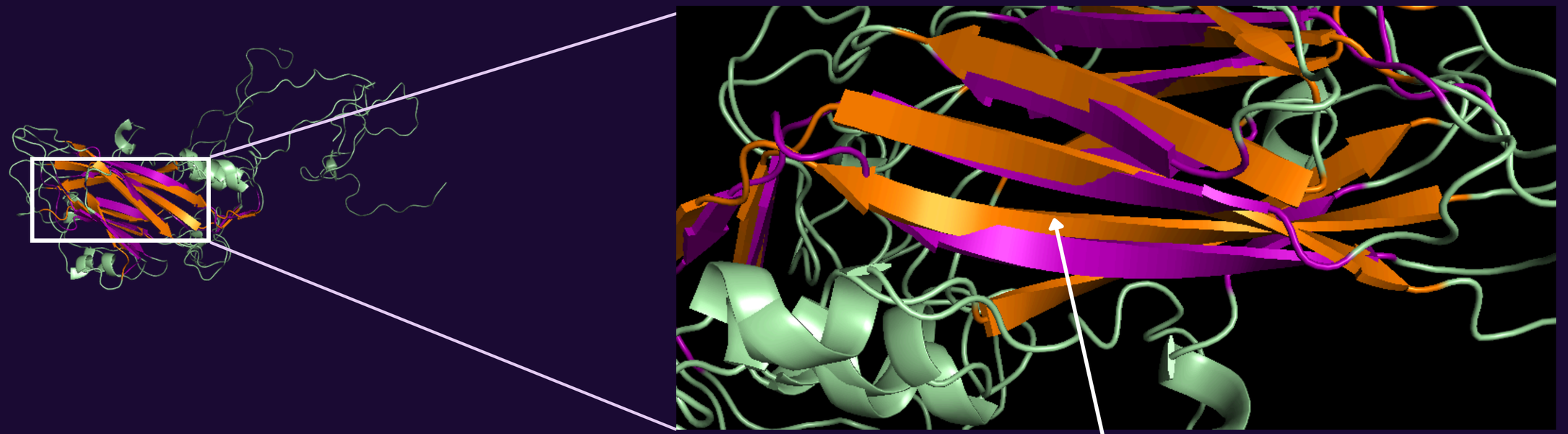


STAMP ALIGNMENT



VP1-VP3 structural comparison

Best superposition regions



VP1

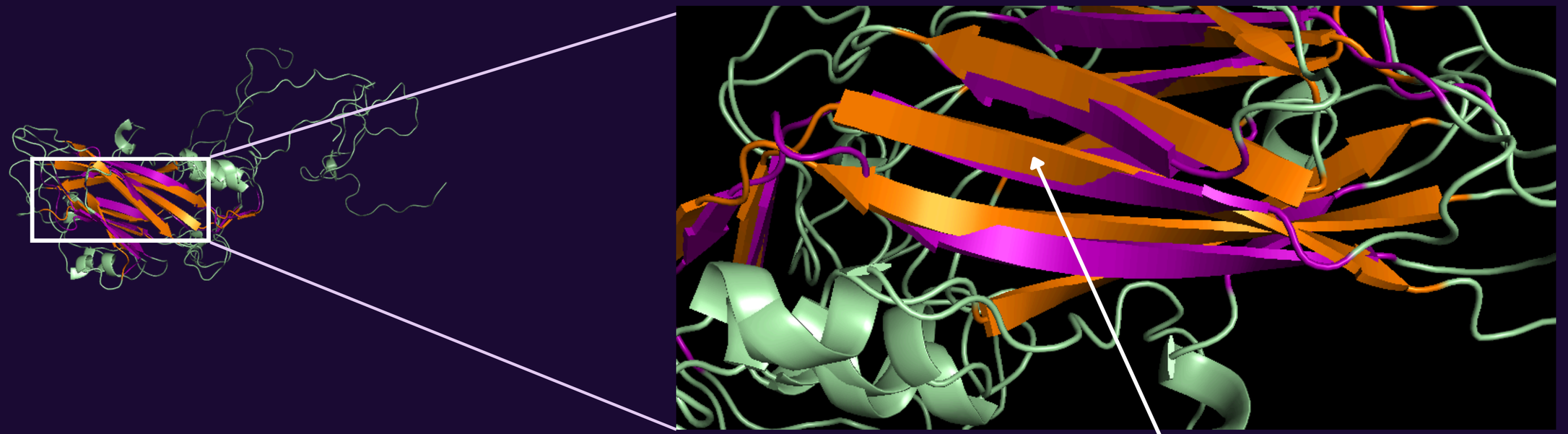
VP3

STAMP ALIGNMENT

```
                  240       250       260       270       280       290
VP1/1-273   HMGSMAFRIVNEHD--E--HKTLVKIRVYHRAK-HVEAWIPRAPR-ALPYTSIGRTNYPKN
VP3/1-236   SAGFLSCWYQTSLILPPETTG-QVYLLSFISACPDFKLRLMKDTQTIS------------
                                  βI
```

VP1-VP3 structural comparison

Best superposition regions

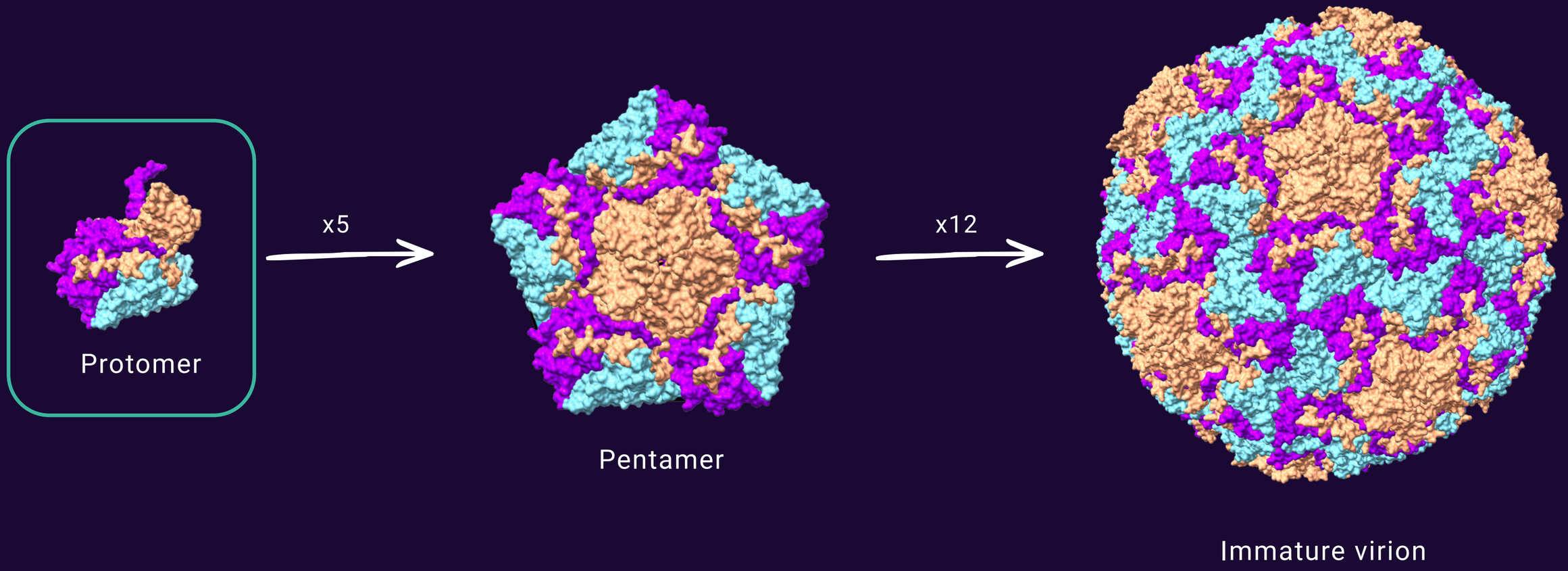


STAMP ALIGNMENT

```
                120       130       140       150       160       17
VP1/1-273   -SS-L-VQ-LRKKLELFTYVRFDSEYTILATASQPDSANY--SSNLVVQAMYVPP
VP3/1-236   GDGVFKTTLLGEIVQYYTHWSGSLRFSLMYTG-------PALSSAKLILAYTPP
                              βD
```

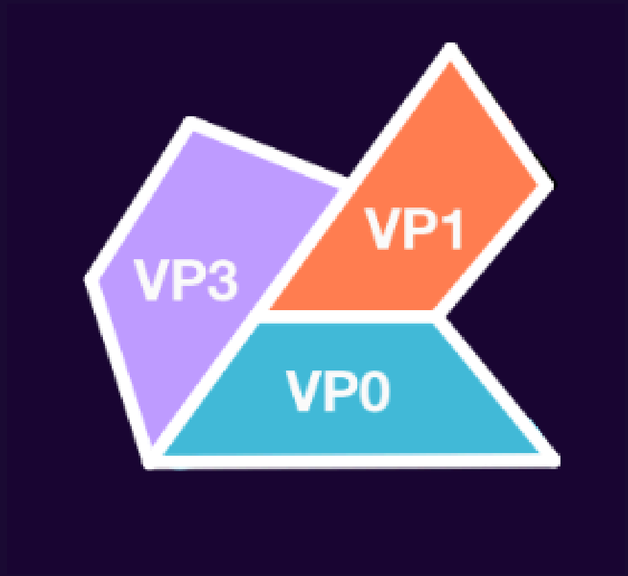
Capsid assembly

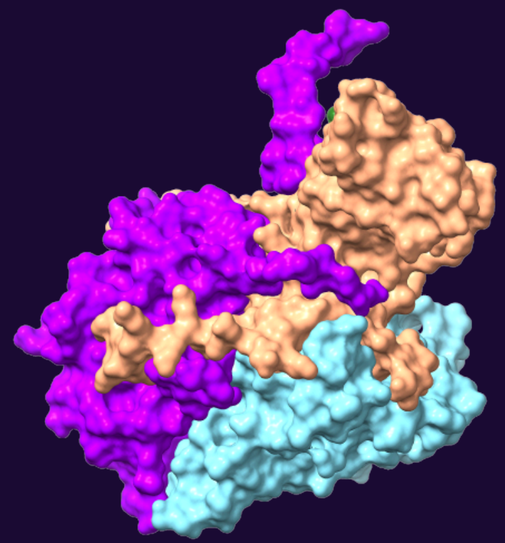
Protomer assembly



Chimera generated

Protomer assembly





Chimera generated

Protomer assembly

Intraprotomer hydrophobic interactions

Table 17

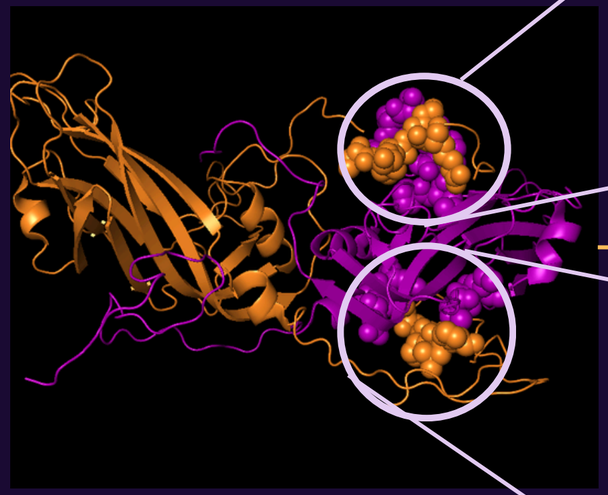
Distribution of types of short non-bonded contacts in HRV14 for interactions within protomers, between protomers in a pentamer and between pentamers

	Hydrophobic	Mixed	Polar	Attractive charged	Total
A. *Within protomer*					
All contacts	251	860	1448	43	2602
Contacts < 3·0 Å	9	69	475	22	579
3·0 Å < d < 3·3 Å	59	313	524	10	909
3·3 Å < d < 3·5 Å	183	478	449	11	1121
B. *Between adjacent protomers (5-fold related in a pentamer)*					
All contacts	15	101	85	4	205
Contacts < 3·0 Å	0	8	23	3	34
3·0 Å < d < 3·3 Å	7	38	28	0	73
3·3 Å < d < 3·5 Å	8	55	34	1	99
C. *Between adjacent pentamers in the HRV14 virion (2-fold and 3-fold related to the reference protomer)*					
All contacts	12	56	66	5	139
Contacts < 3·0 Å	0	5	27	3	35
3·0 Å < d < 3·3 Å	3	14	16	2	35
3·3 Å < d < 3·5 Å	9	37	23	0	69

Analysis of the structure of a common cold virus, human rhinovirus 14, refined at a resolution of 3.0 Å - ScienceDirect

Protomer assembly

Intraprotomer salt bridge







BSA

1:THR 275		49.07	0.67 |	0.01
1:GLU 276		141.16	0.24 |	-0.00
1:PRO 277		120.63	53.72 |||||	0.86
1:VAL 278	H	88.17	32.79 ||||	0.39
1:ILE 279	H	151.52	113.06 ||||||||	1.78
1:LYS 280	S	199.60	95.59 |||||	0.50
1:LYS 281	H	189.95	77.77 |||||	-0.34
1:ARG 282	H	112.69	90.64 |||||||||	-0.97

VP1

3:PRO 54		22.62	20.79 |||||||||	0.33
3:MET 55	H	13.53	13.53 |||||||||	-0.01
3:ASN 56		29.22	12.82 |||||	-0.03
3:ASN 57	H	87.96	77.74 ||||||||	-0.42
3:THR 58	H	109.10	65.72 ||||||	0.66
3:HIS 59		67.69	18.92 |||	-0.20
3:THR 60		139.02	28.89 |||	-0.07
3:LYS 61		136.78	16.68 ||	0.27
3:ASP 62	H	147.68	89.93 ||||||	-0.61
3:GLU 63		55.85	1.40 |	-0.02

VP3

1:ALA 41		46.14	1.35 |	-0.02
1:THR 42	H	133.74	93.68 |||||||	0.81
1:MET 43		102.12	10.16 |	-0.08
1:PRO 44		103.23	14.88 ||	0.24
1:VAL 45	H	67.30	63.30 |||||||||	0.35
1:LEU 46		102.49	37.15 ||||	0.59
1:PRO 47		90.21	81.75 |||||||||	1.31

VP1

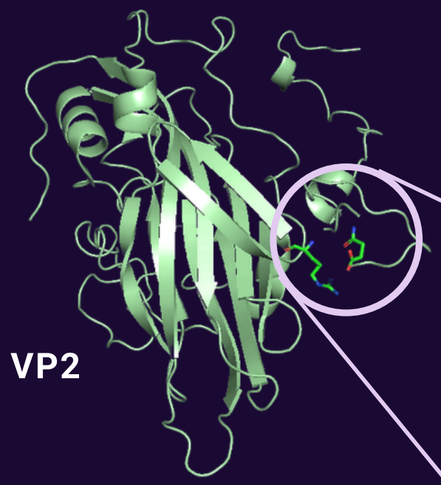
3:LEU 157		170.58	0.61 |	-0.01
3:GLN 158		147.25	63.16 |||||	0.52
3:SER 159	H	40.99	17.62 |||||	0.14
3:THR 160	H	53.69	53.69 |||||||||	0.13
3:ILE 161		29.35	25.67 ||||||||	0.41
3:VAL 162	H	81.80	77.78 |||||||||	0.65
3:MET 163		1.70	0.69 |||||	0.01
3:THR 164		62.26	61.01 |||||||||	0.66

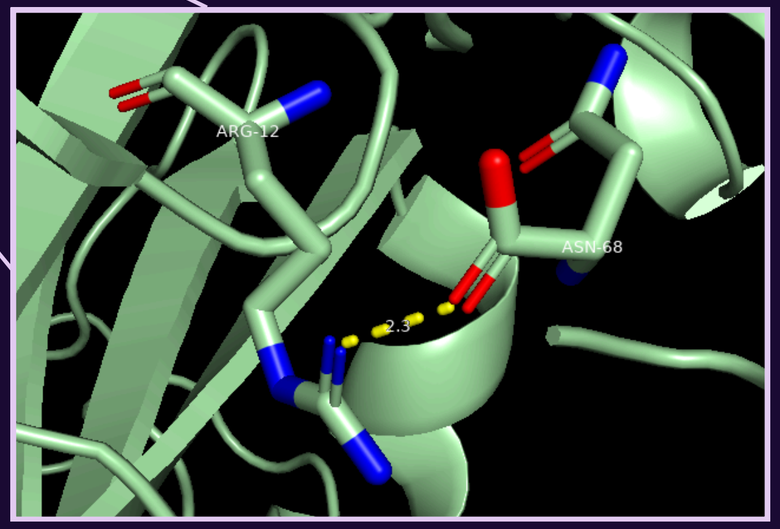
VP3

Pymol generated

Protomer assembly

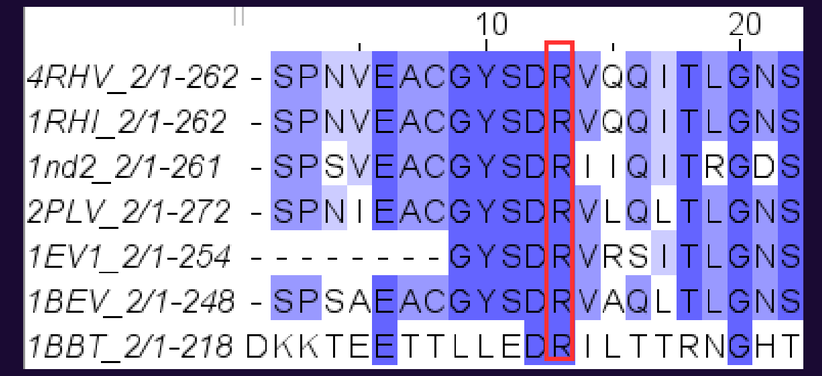
Intraprotomer salt bridge





ARG 12 (VP2) - ASN 68 (C-TERM,VP4)

ARG 12 (VP2)



ASN 68 (C_TERM VP4)



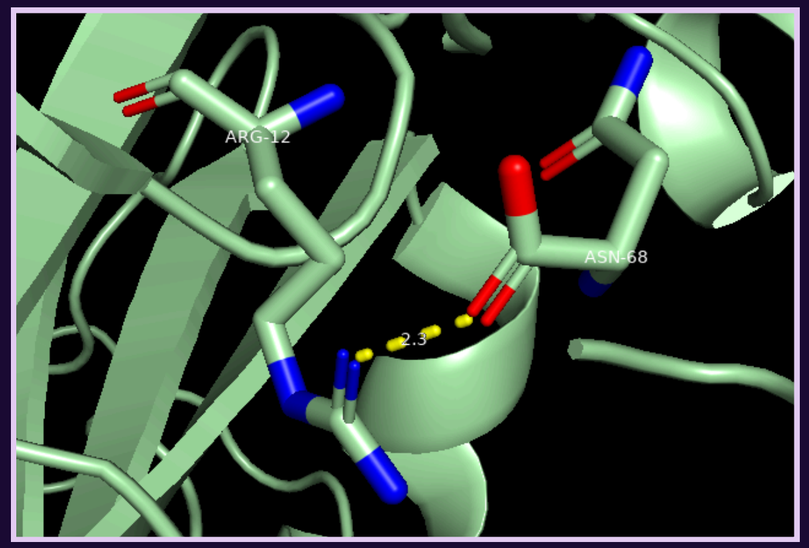
4RHV: *Human Rhinovirus B14*
1RHI: *Human Rhinovirus B3*
1ND2: *Human Rhinovirus A16*
2PLV: *Human Poliovirus 1*
1EV1: *Echovirus 1*
1BEV: *Bovine Rhinovirus*
1BBT: *Foot and Mouth virus VG-5-27*

Pymol generated

Protomer assembly

Intraprotomer salt bridge

ARG 12 (VP2) - ASN 68 (C-TERM,VP4)



The distance of this interaction is 2.3 Å

This interaction takes place between:

ARG 12 (VP2) :

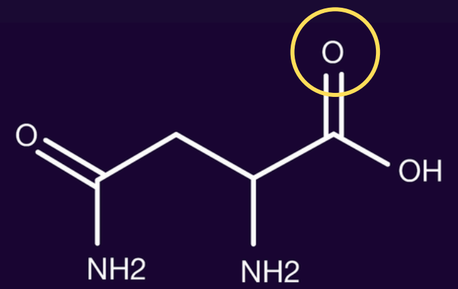
- Located in the βA1.
- Interacts through the NH of the guanidine group.

ASN 68 (C-TERM, VP4):

- Located in the VP4 loop (C-term).
- Interacts through the O of its carboxyl group (COOH).



Arginine



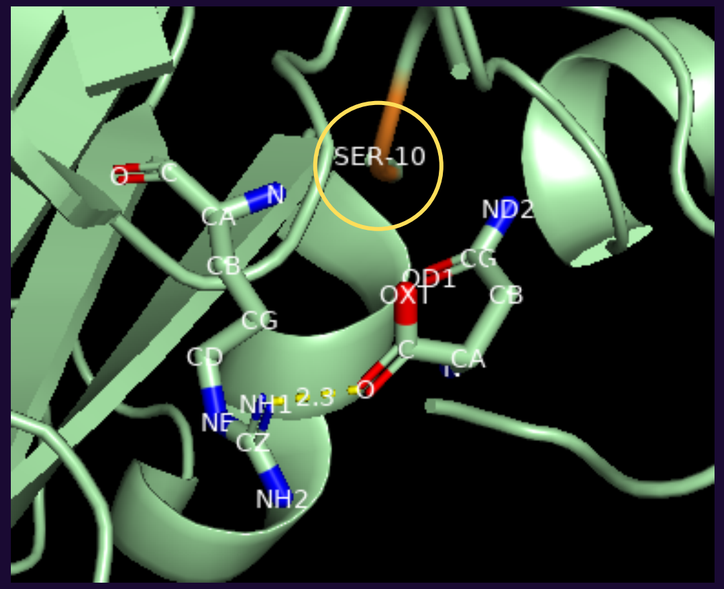
Asparagnina

Pymol generated

Protomer assembly

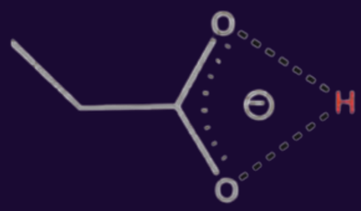
Intraprotomer salt bridge

ARG 12 (VP2) - ASN 68 (C-TERM,VP4)



Significance in VP0 autocleavage

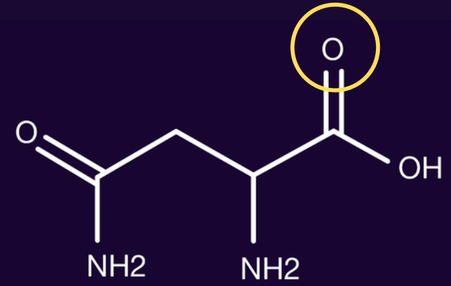
- RNA acts as catalys by converting the Ser 2010 hydroxyl to the more nucleophilic alkoxide form.
- This cleavage requires the formation of a **tetrahedral oxyanion** intermediate via a nucleophilic attack by the Ser2010 alkoxide.
- This interaction could provide some stabilization of negative charge that occur during the autocatalytic cleavage



Tetrahedral oxyanion intermediate



Arginine

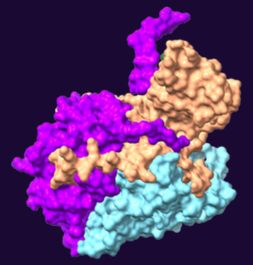


Asparagnina

Pymol generated

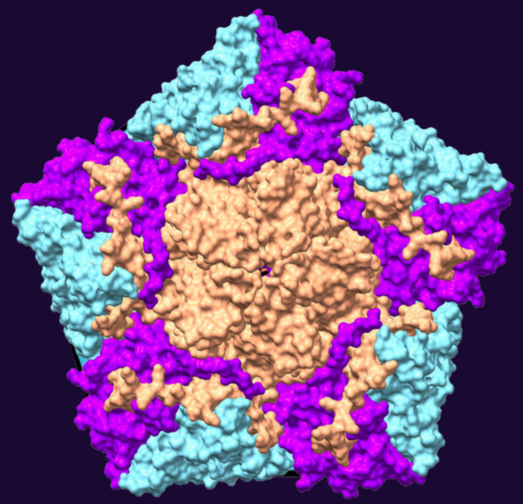
Capsid assembly

Pentamer assembly



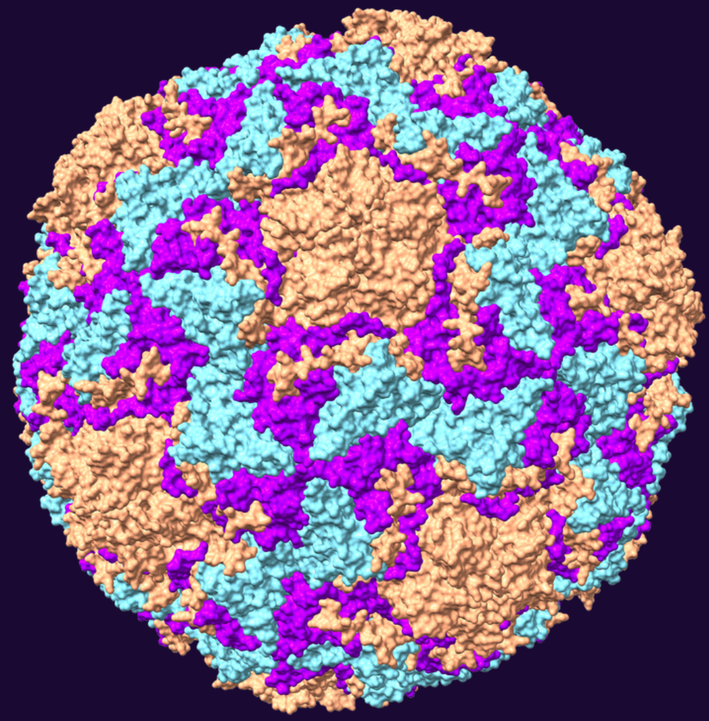
Protomer

x5



Pentamer

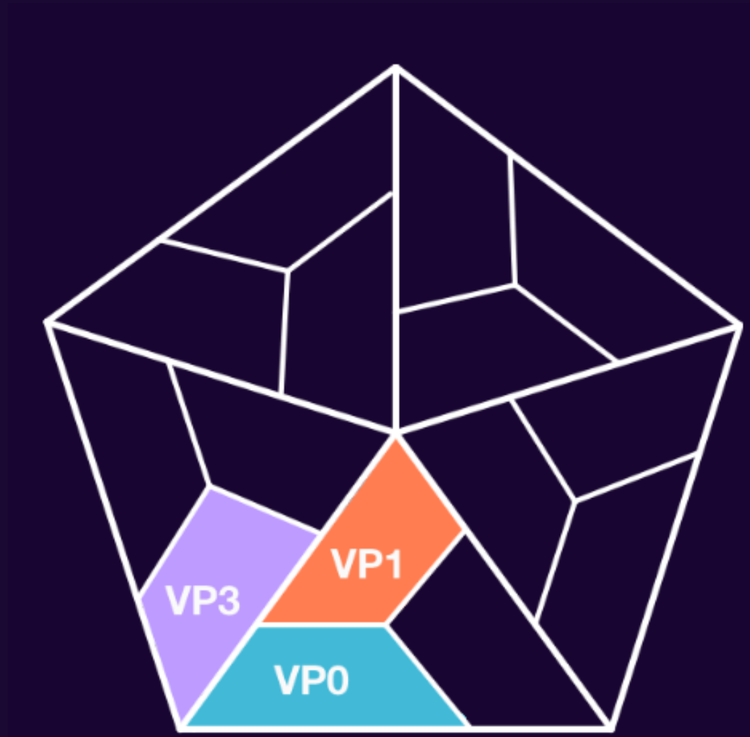
x12

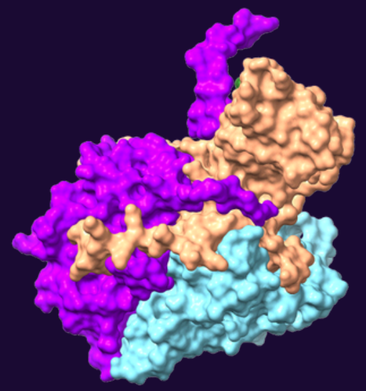


Immature virion

Chimera generated

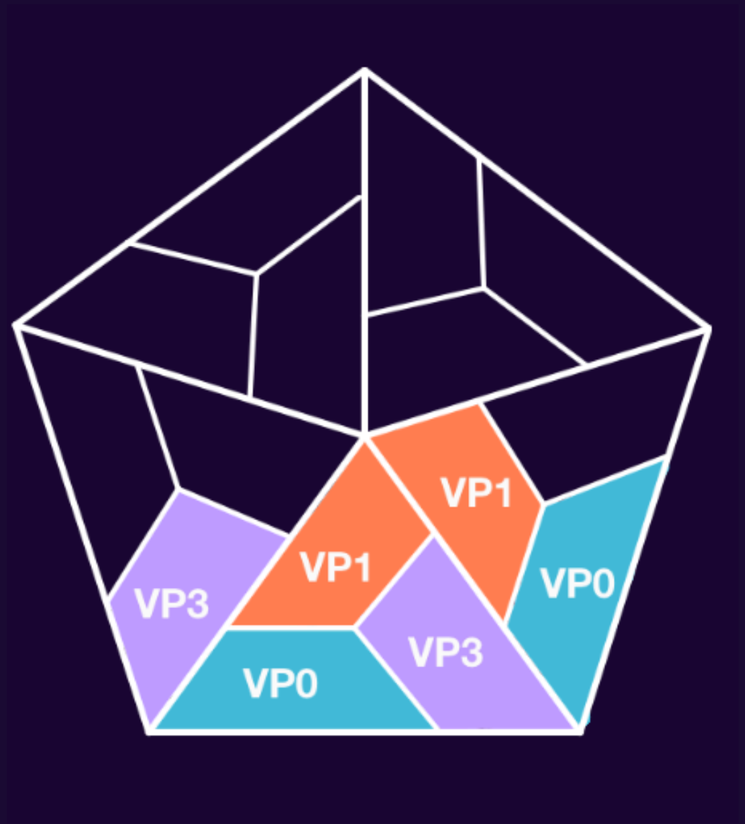
Pentamer assembly

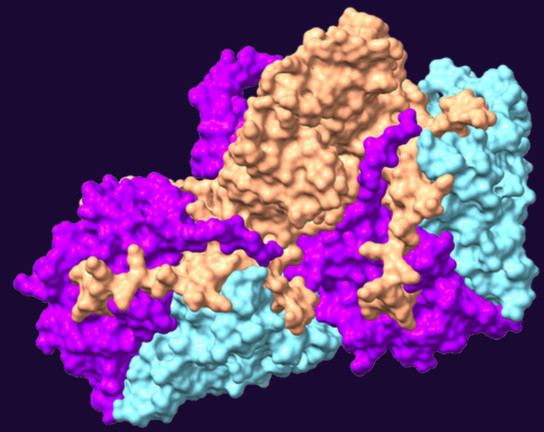




Chimera generated

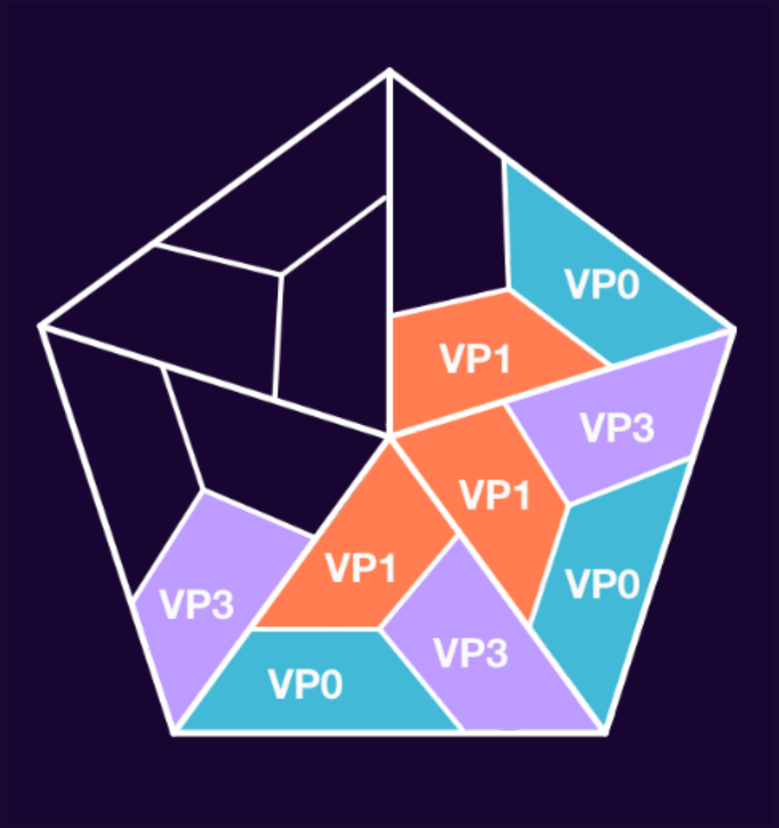
Pentamer assembly

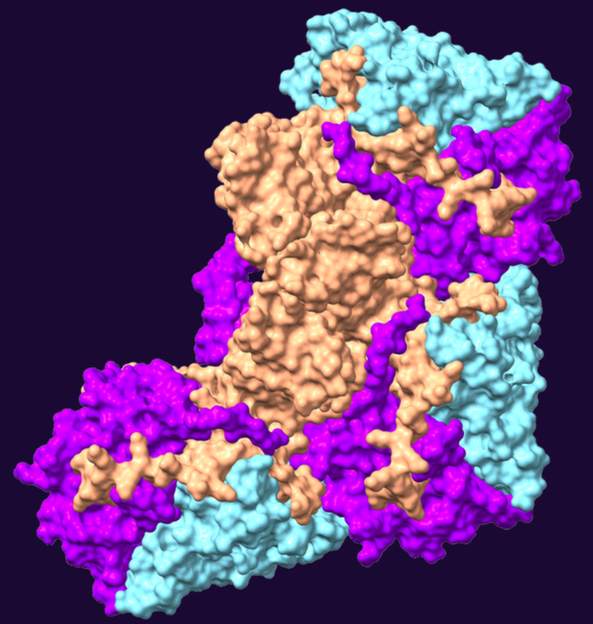




Chimera generated

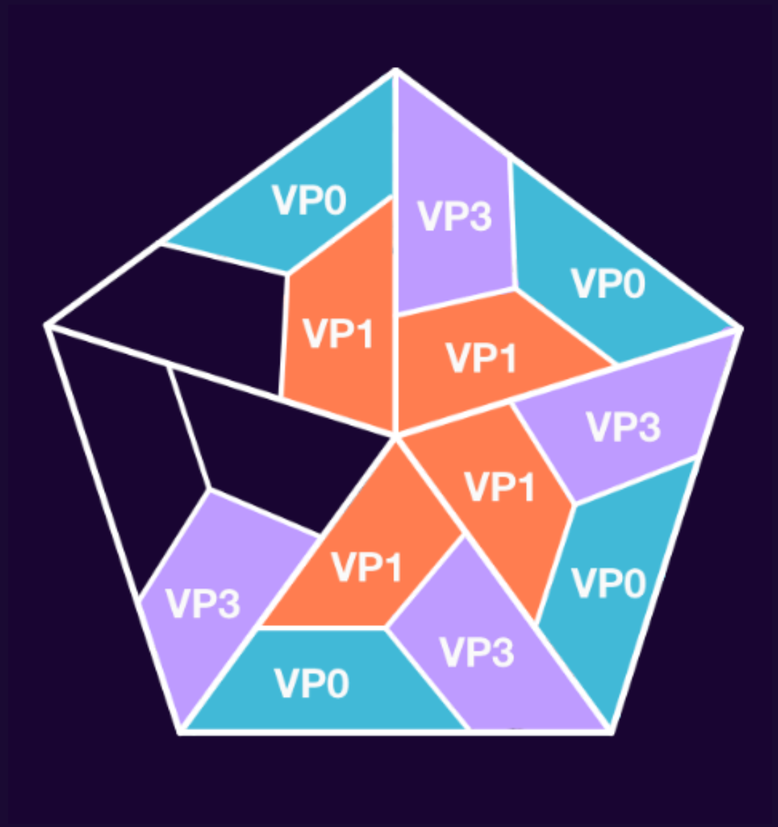
Pentamer assembly

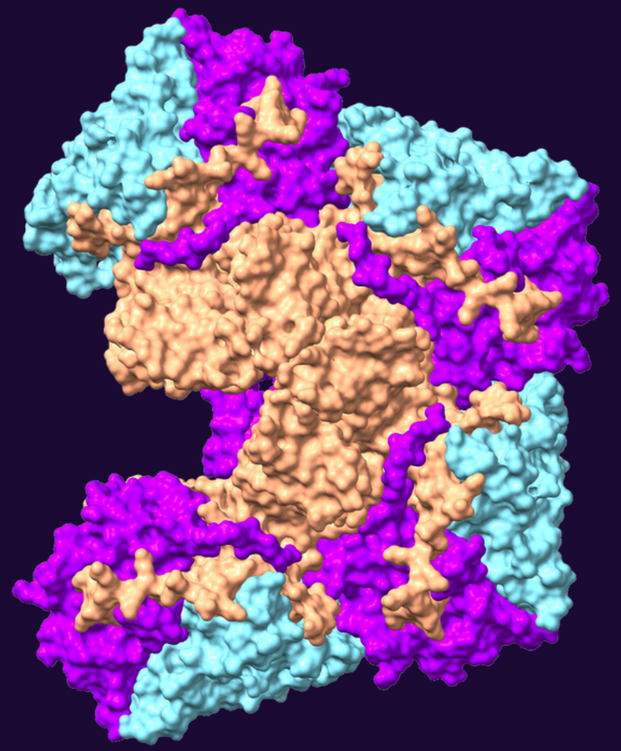




Chimera generated

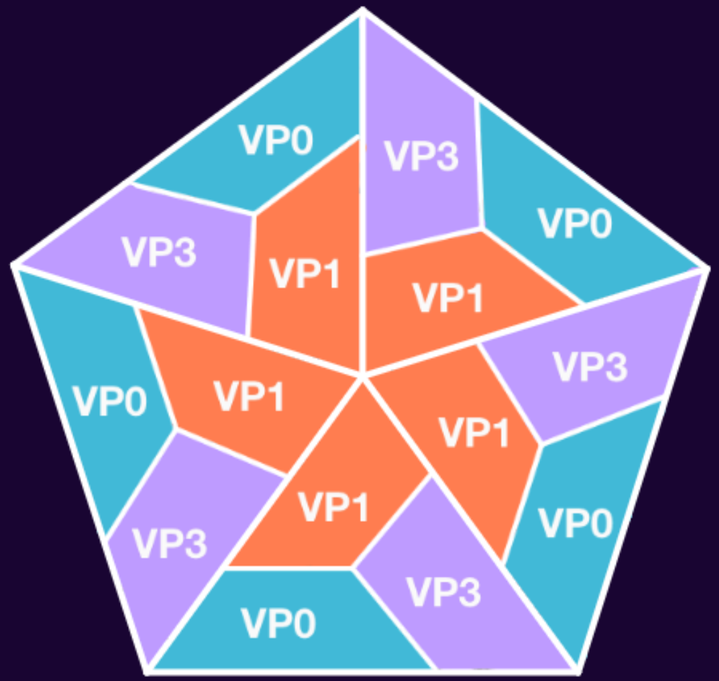
Pentamer assembly

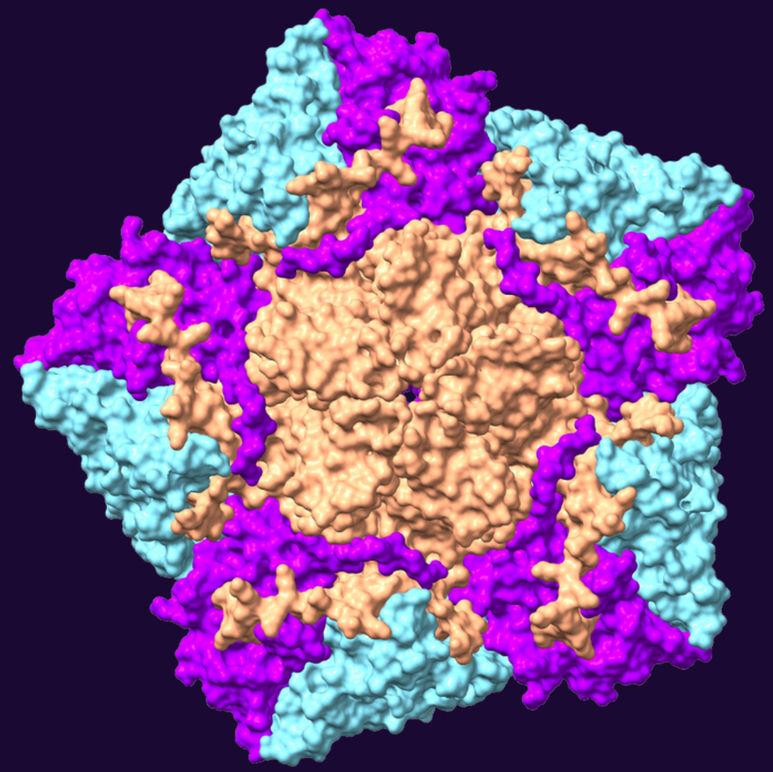




Chimera generated

Pentamer assembly

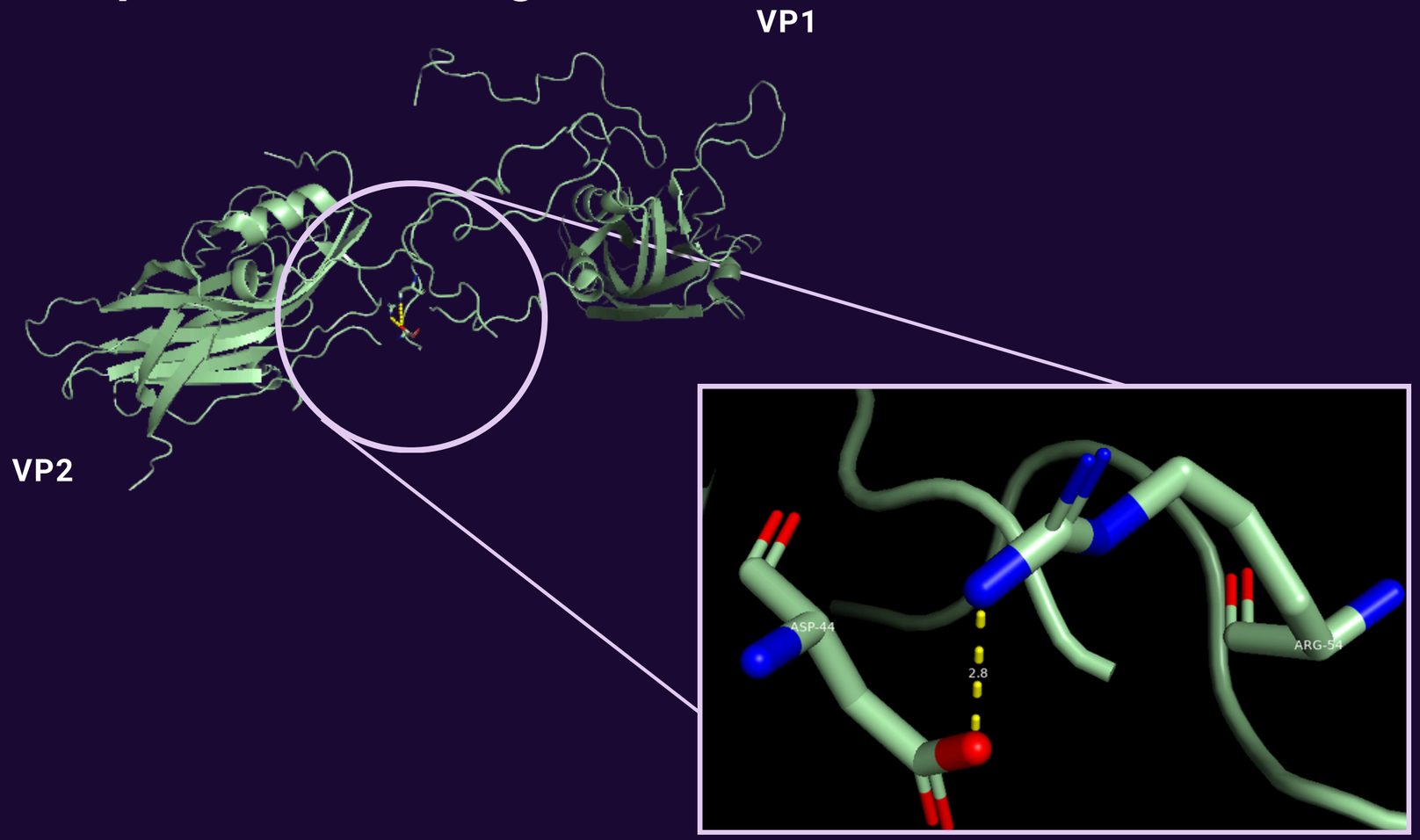




Chimera generated

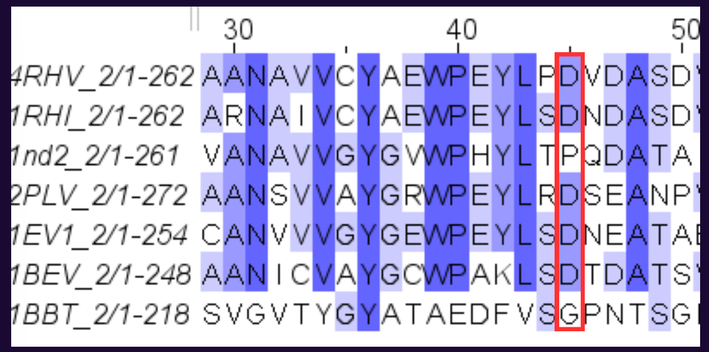
Pentamer assembly

Interprotomer salt bridge

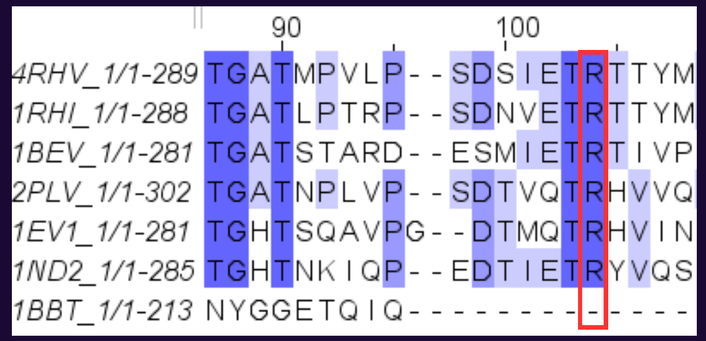


ASP 44 (VP2) - ARG 54 (VP1)

ASP 44 (VP2)



ARG 54 (VP1)



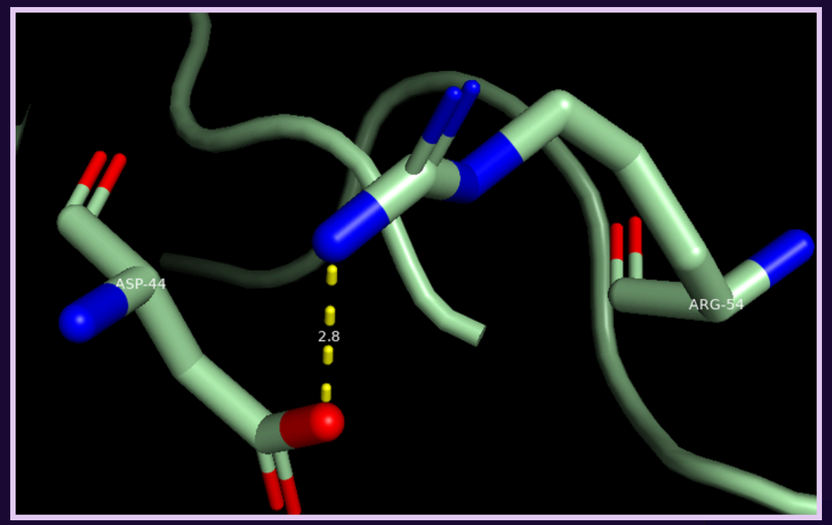
4RHV: *Human Rhinovirus B14*
1RHI: *Human Rhinovirus B3*
1ND2: *Human Rhinovirus A16*
2PLV: *Human Poliovirus 1*
1EV1: *Echovirus 1*
1BEV: *Bovine Rhinovirus*
1BBT: *Foot and Mouth virus VG-5-27*

Pymol generated

Pentamer assembly

Interprotomer salt bridge

ASP 44 (VP2) - ARG 54 (VP1)



The distance of this interaction is 2.8 Å

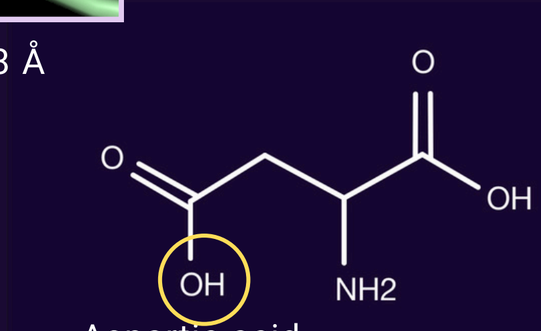
This interaction takes place between:

ASP 44 (VP2) :

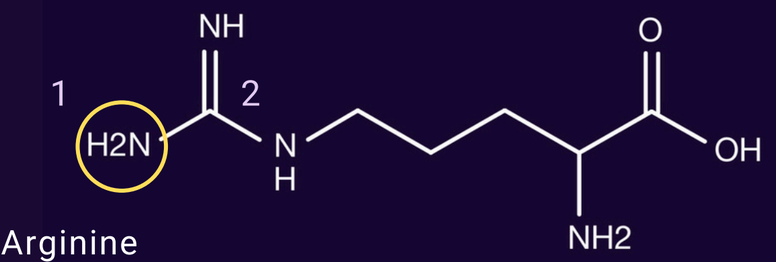
- Located in a loop between βA2 and αZ helix
- Interacts through its carboxyl group (COOH).

ARG 54(VP1):

- Located in the N-term arm.
- Interacts through the NH2 group of its guanadine.



Aspartic acid

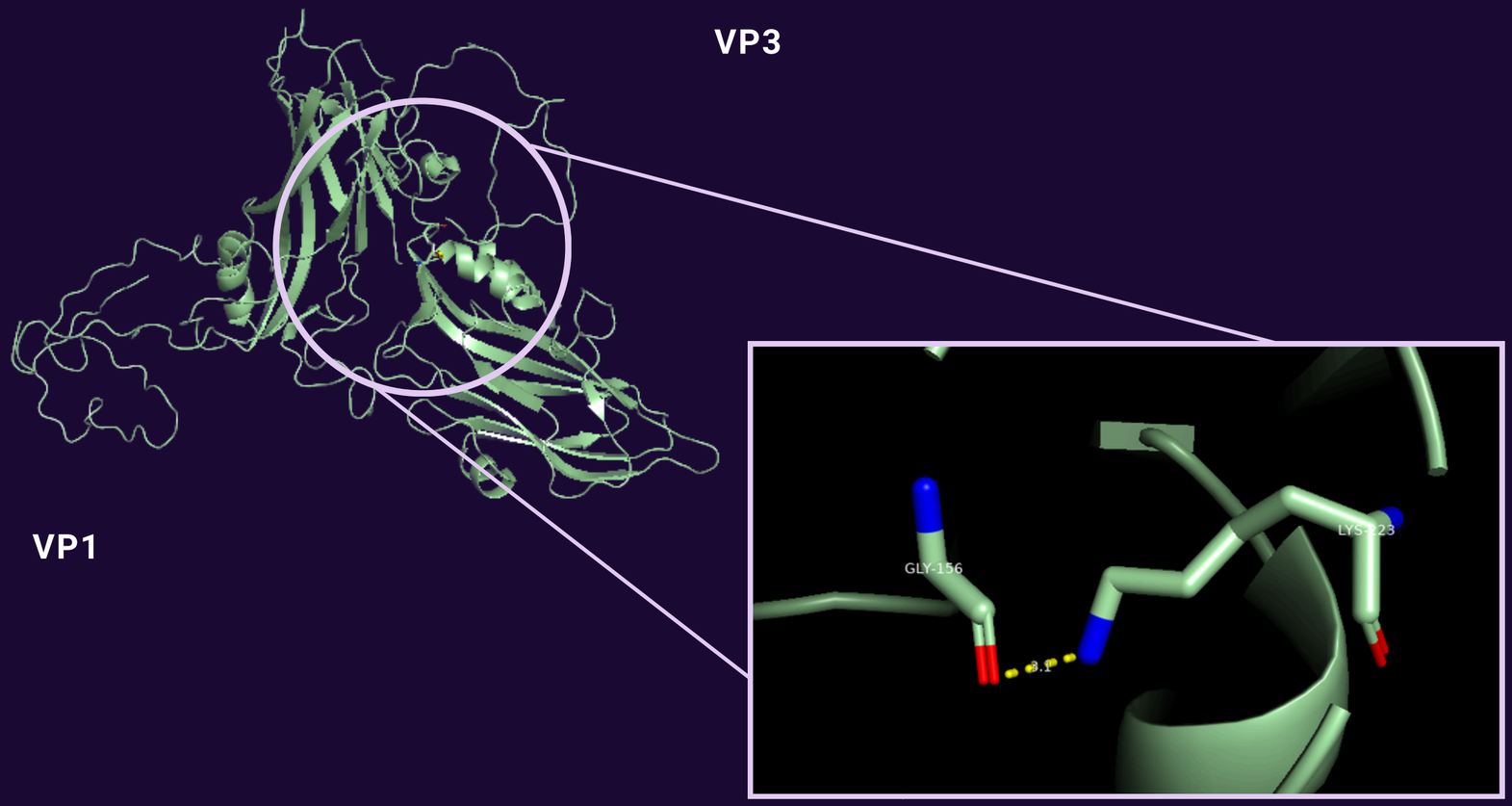


Arginine

Pymol generated

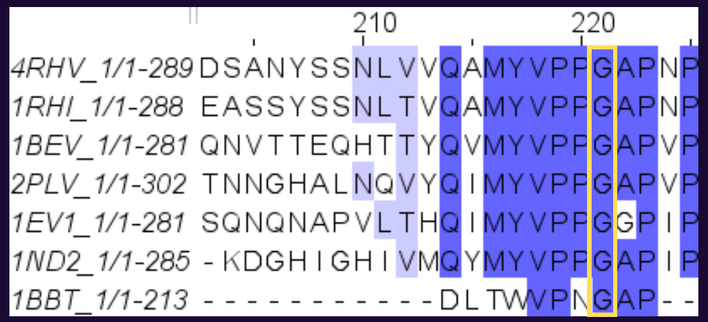
Pentamer assembly

Interprotomer polar interaction

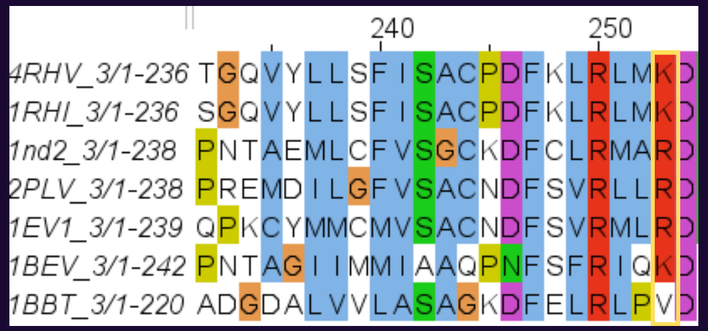


GLY 156 (VP1) - LYS 223 (VP3)

GLY 156 (VP1)



LYS 223 (VP3)



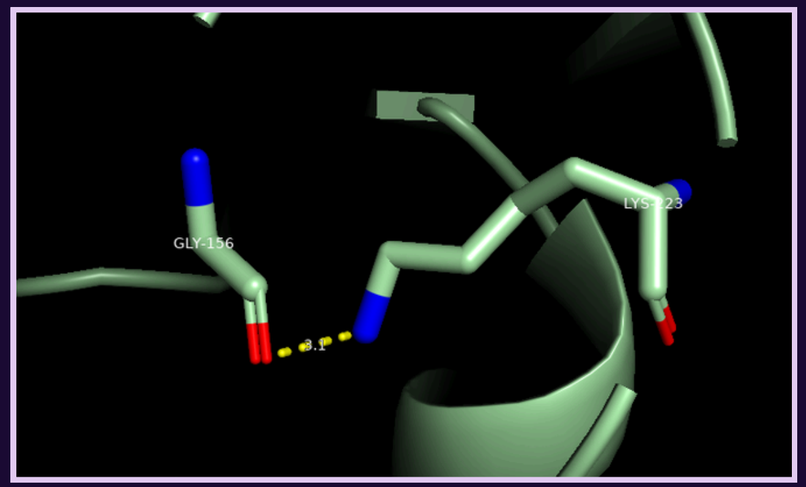
4RHV: *Human Rhinovirus B14*
1RHI: *Human Rhinovirus B3*
1ND2: *Human Rhinovirus A16*
2PLV: *Human Poliovirus 1*
1EV1: *Echovirus 1*
1BEV: *Bovine Rhinovirus*
1BBT: *Foot and Mouth virus VG-5-27*

Pymol generated

Pentamer assembly

Interprotomer polar interaction

GLY 156 (VP1) - LYS 223 (VP3)



The distance of this interaction is 3.1 Å

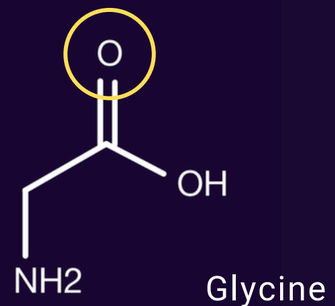
This interaction takes place between:

GLY 156 (VP1) :

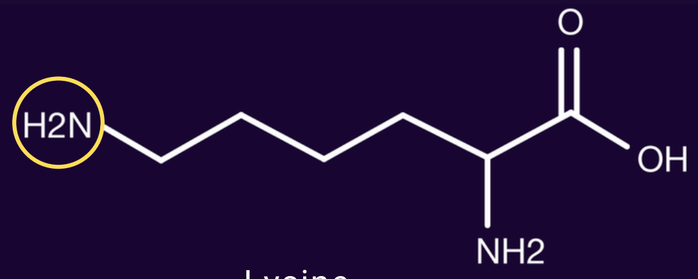
- Located in the loop between βE and αB.
- Interacts through its carboxyl group (COOH).

LYS 223 (VP3):

- Located in the C-term tail.
- Interacts through the NH2 group of its side chain.



Glycine

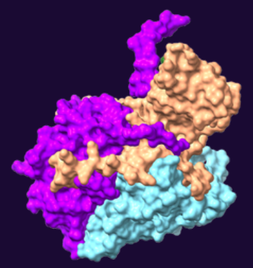


Lysine

Pymol generated

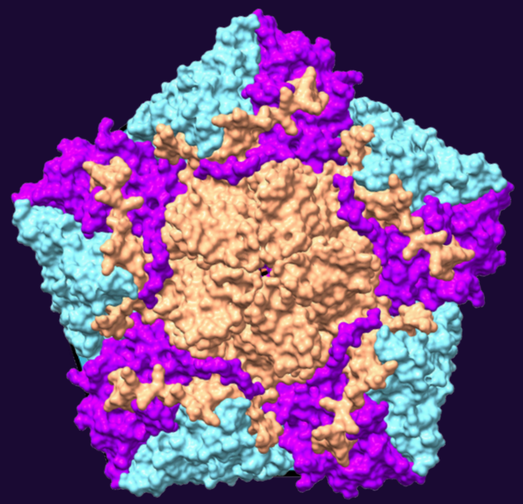
Capsid assembly

Immature virion: Pentamer binding



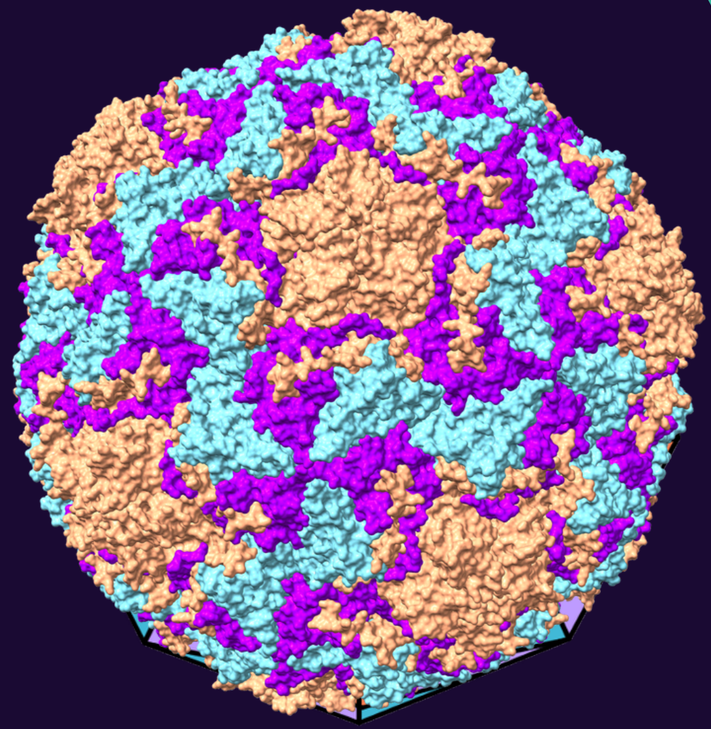
Protomer

x5



Pentamer

x12

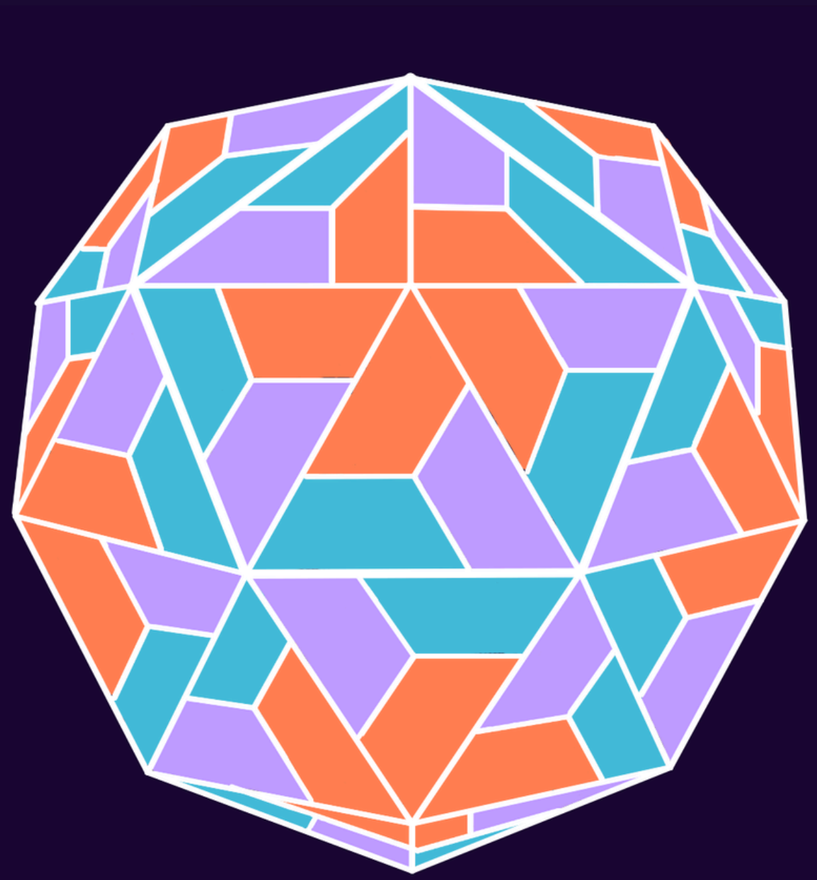


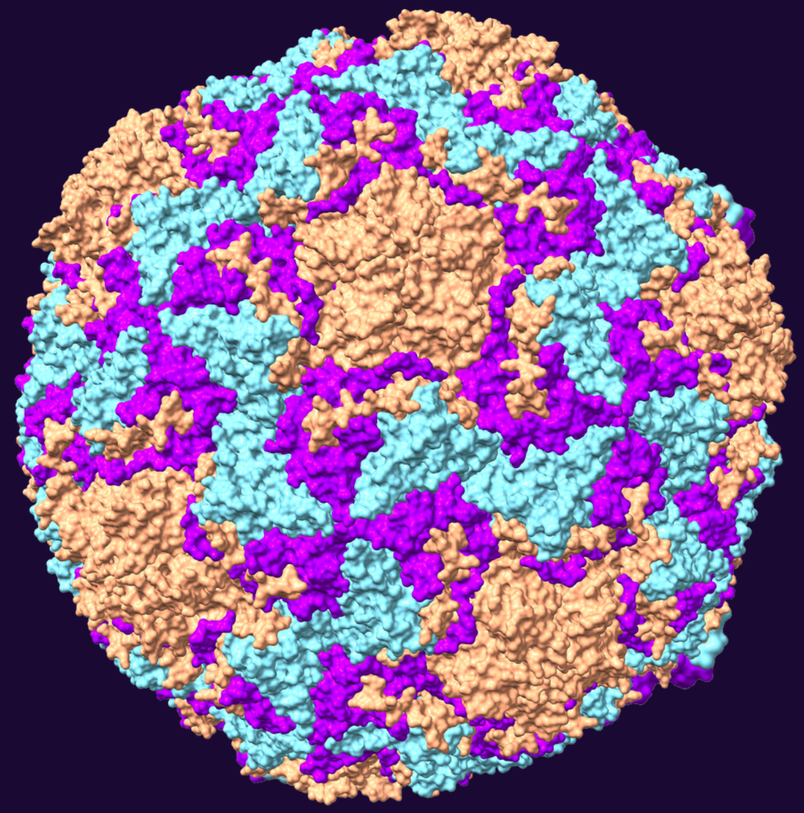
Immature virion

Chimera generated

Capsid assembly

Immature virion: Pentamer binding





VP1 VP0 VP3

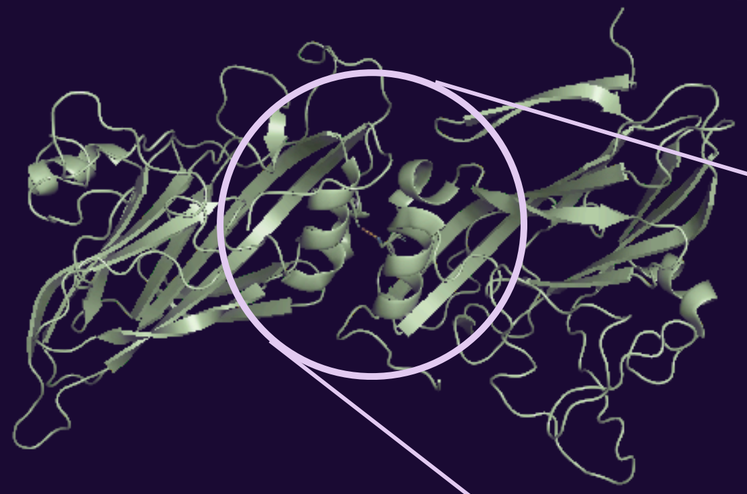
Chimera generated

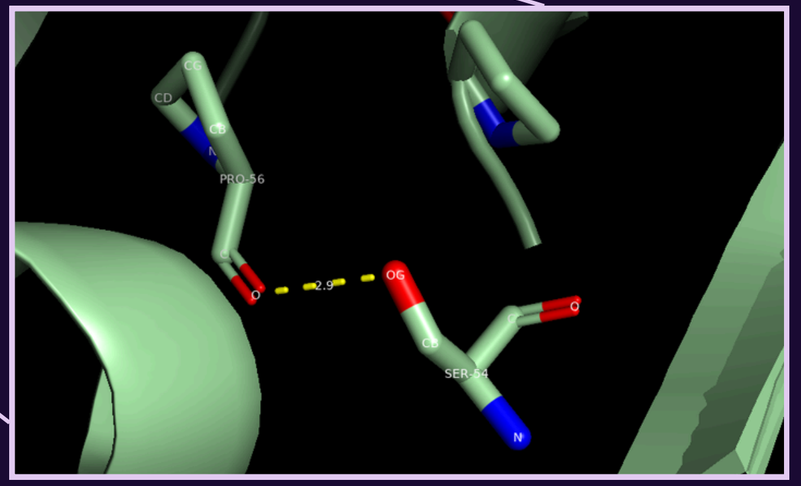
Immature virion: Pentamer binding

Interpentamer polar interaction

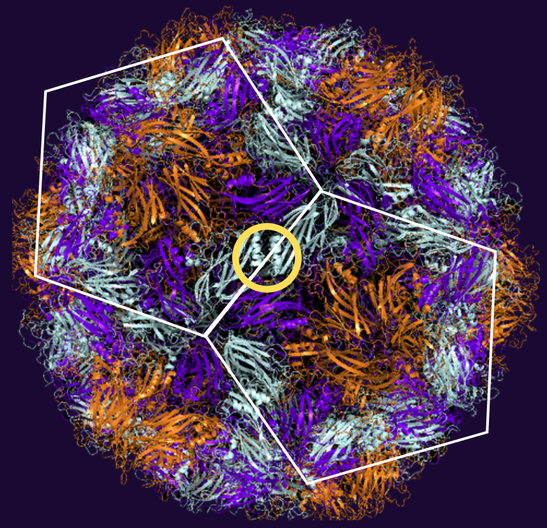
VP2

VP2





PRO 56 (VP2) - SER 54 (VP2)

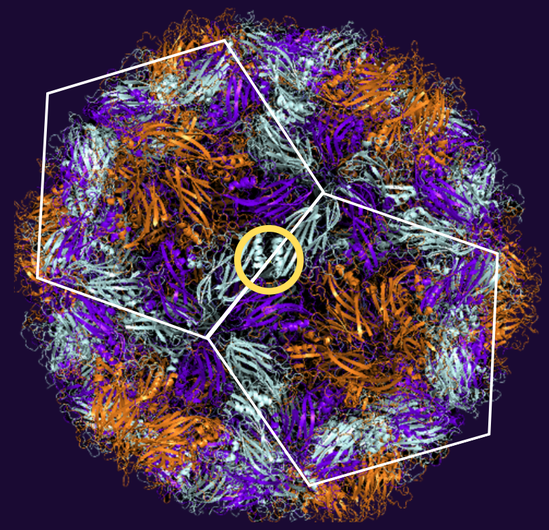


2-fold related

Pymol generated

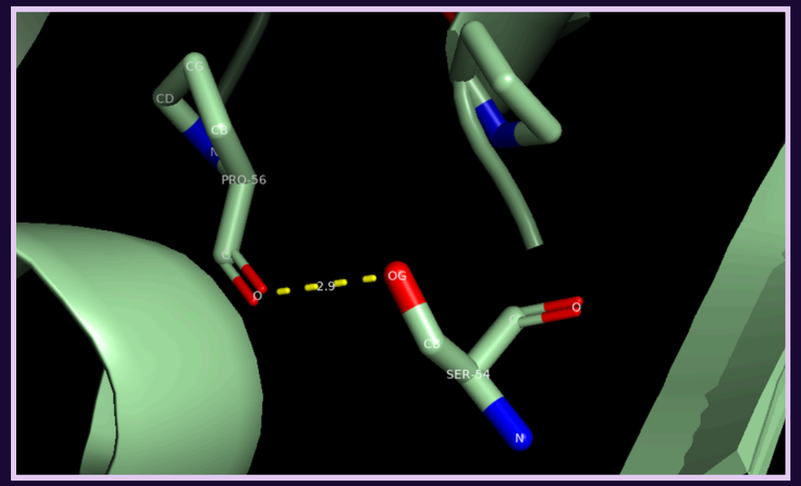
Immature virion: Pentamer binding

Interpentamer polar interaction



2-fold related

PRO 56 (VP2) - SER 54 (VP2)



The distance of this interaction is 2.9 Å

This interaction takes place between:

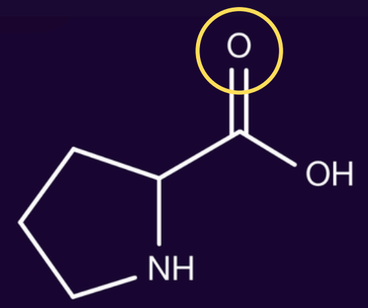
PRO 56 (VP2):

- Located in the αZ helix.
- Interacts through its carboxyl group (COOH).

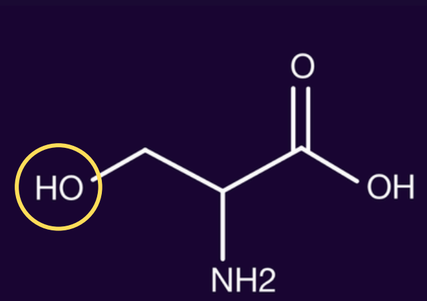
SER 54 (VP2):

- Located in the loop between βA2 and αZ helix.
- Interacts through the hidroxil group (OH) of its side chain.





Proline

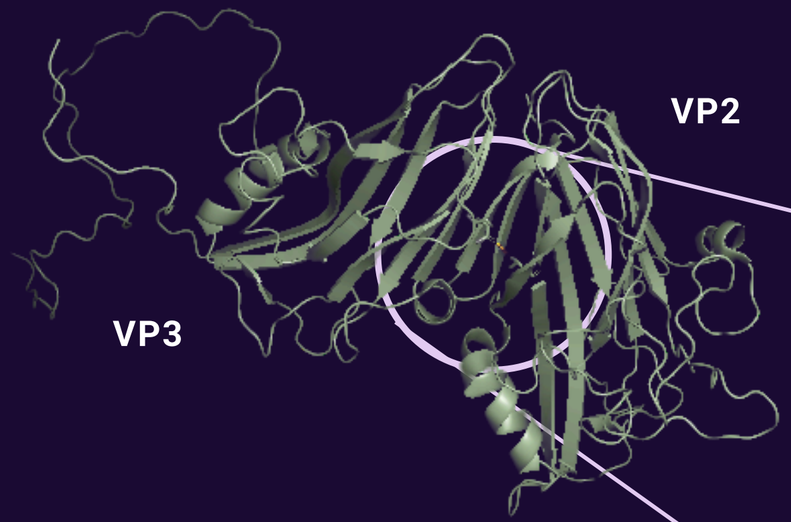


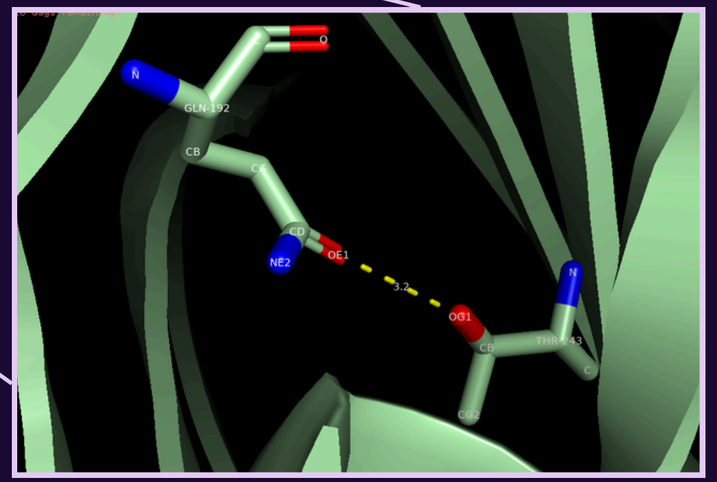
Serine

Pymol generated

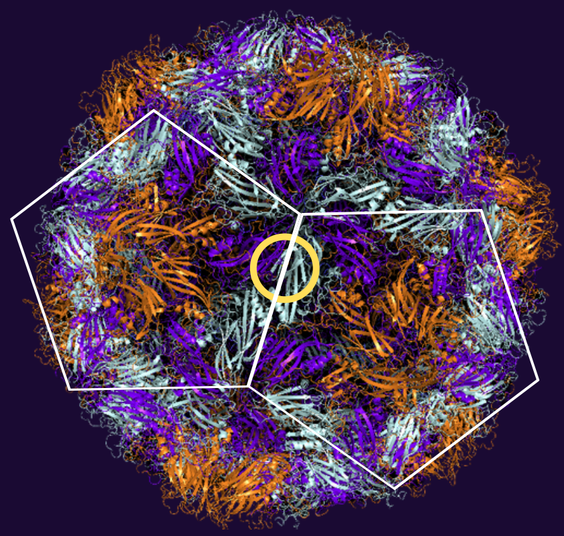
Immature virion: Pentamer binding

Interpentamer polar interaction





GLN 192 (VP3) - THR 243 (VP2)



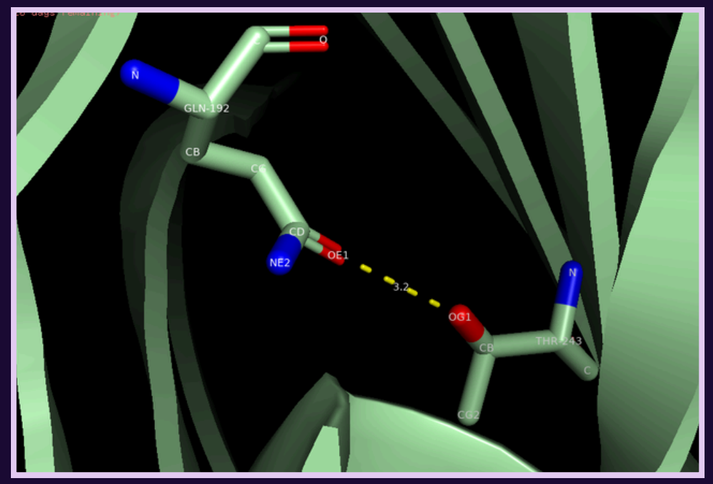
3-fold related

Pymol generated

Immature virion: Pentamer binding

Interpentamer polar interaction

GLN 192 (VP3) - THR 243 (VP2)



The distance of this interaction is 3.2 Å

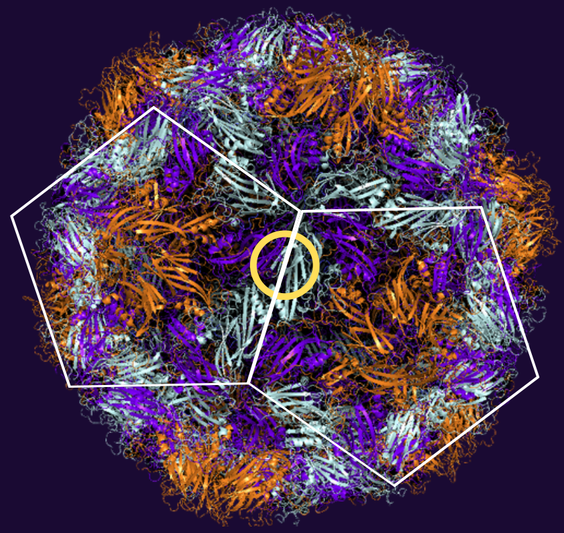
The interaction takes place between:

GLN 192 (VP3):

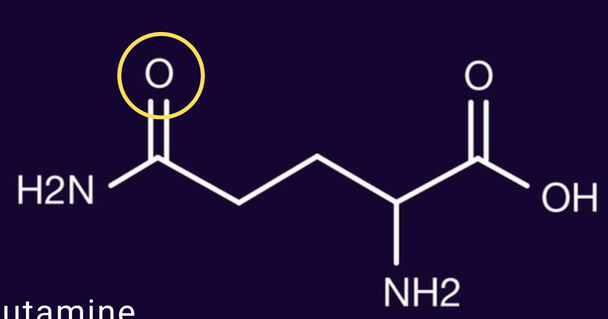
- Located in the βH.
- Interacts through the amide group ($C(O)NH_2$) of its side chain.

THR 243 (VP2):

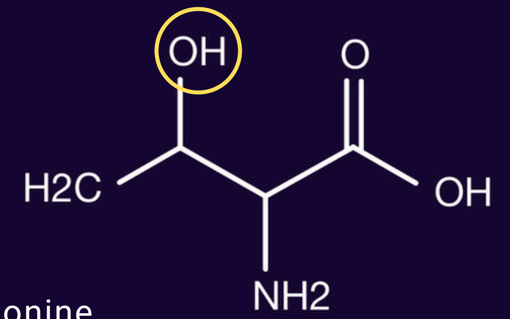
- Located in the βI.
- Interacts through the hydroxyl group (OH) of its side chain.



3-fold related



Glutamine

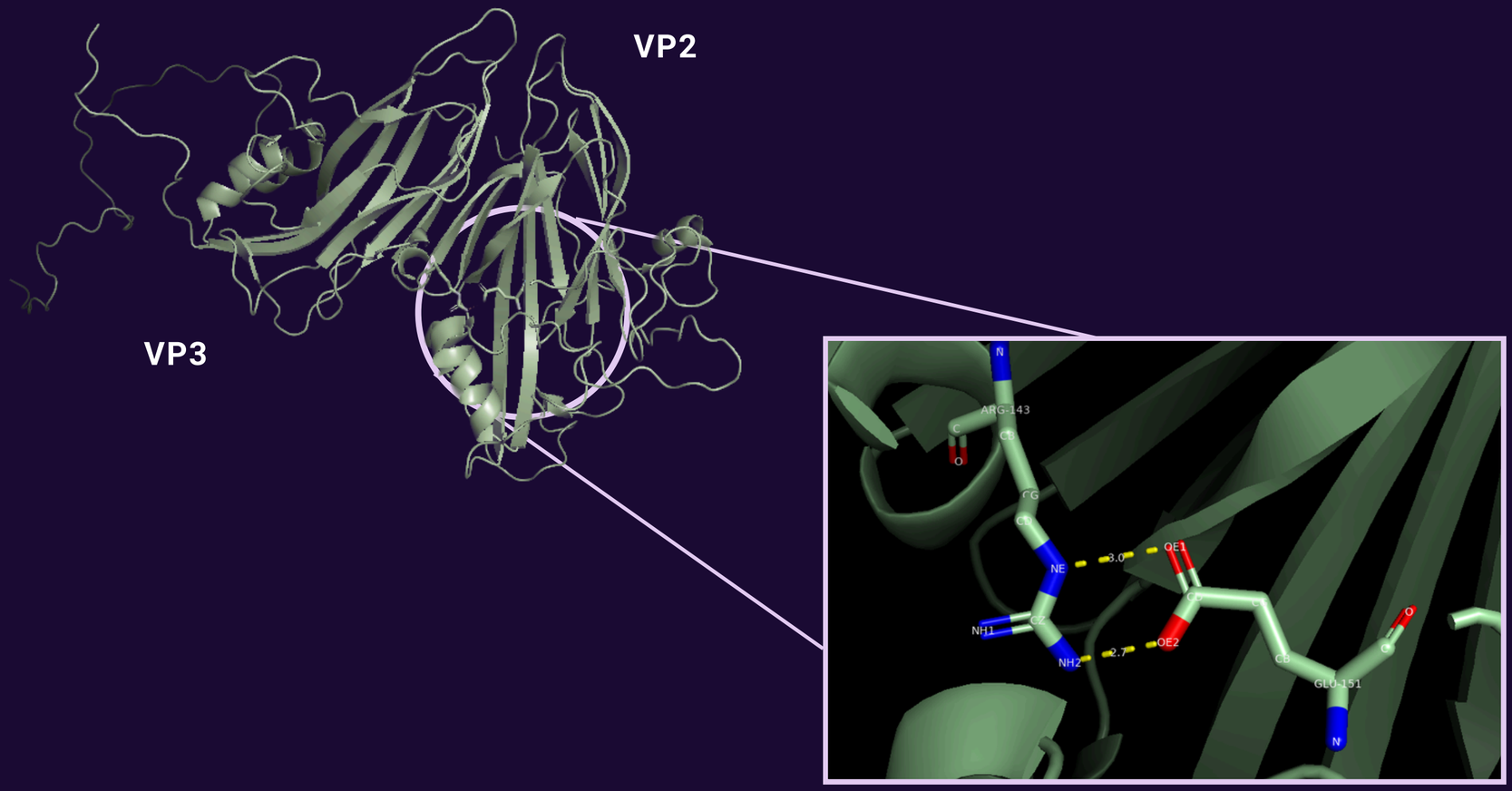


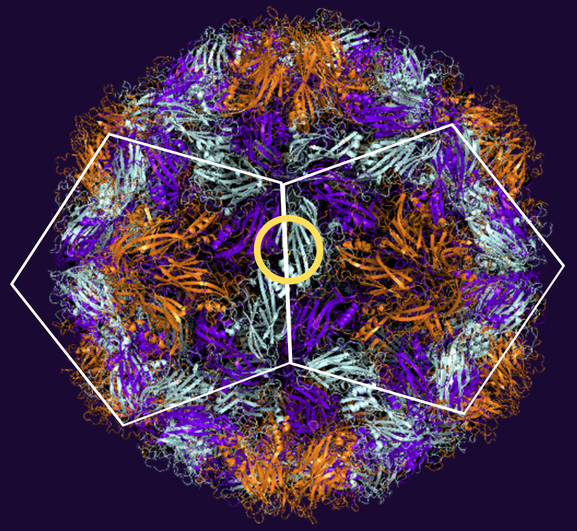
Threonine

Pymol generated

Immature virion: Pentamer binding

Interpentamer salt bridge

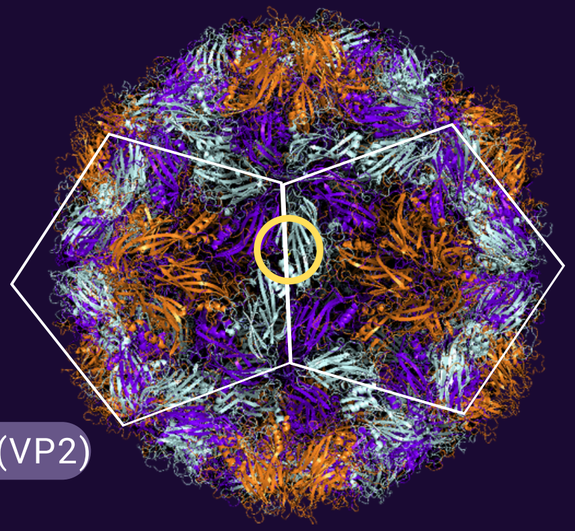




ARG 143 (VP3) - GLU 151 (VP2)

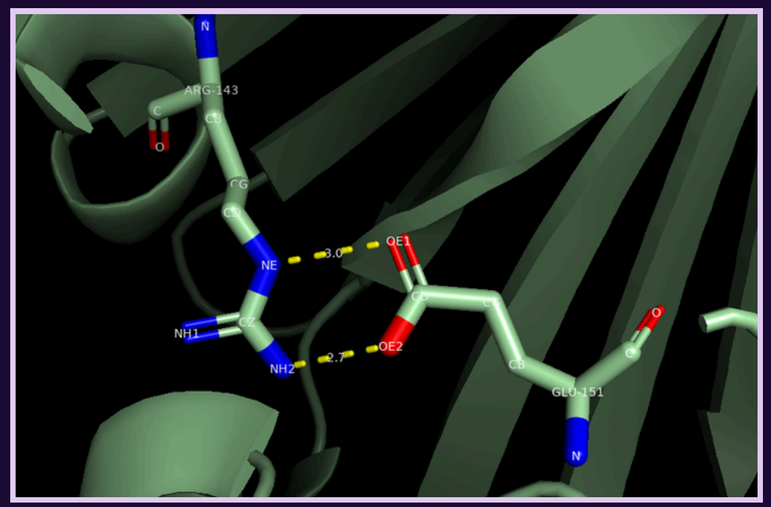
Pymol generated

Immature virion: Pentamer binding



Interpentamer salt bridge

ARG 143 (VP3) - GLU 151 (VP2)

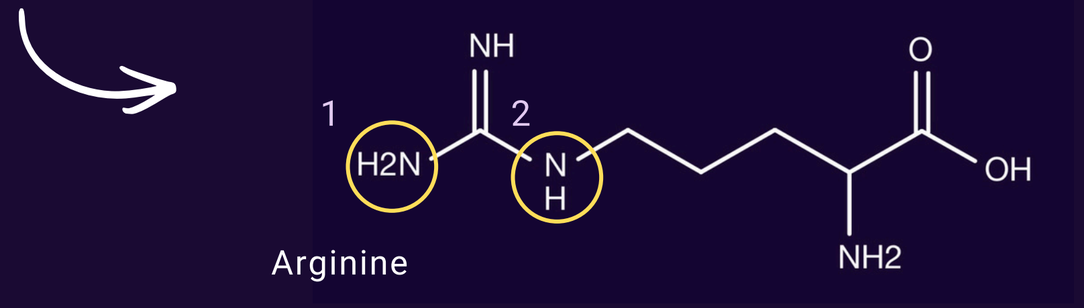


The interaction takes place between:

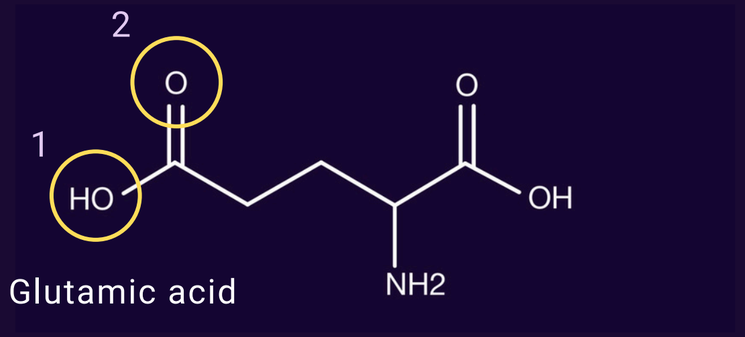
ARG 143 (VP3) located in αB, interacts with GLU 151 (VP2) located in the PUFF loop.

1. The interaction between the amine group (NH2) of the guanidine in the arginine and the OH of the carboxyl group (COOH) in the glutamic acid is at a distance of 2.7 Å.

2. The interaction between the NH group of the guanidine in the arginine and the O of the carboxyl group (COOH) in the glutamic acid side chain is at a distance of 3.0 Å.



Arginine



Glutamic acid

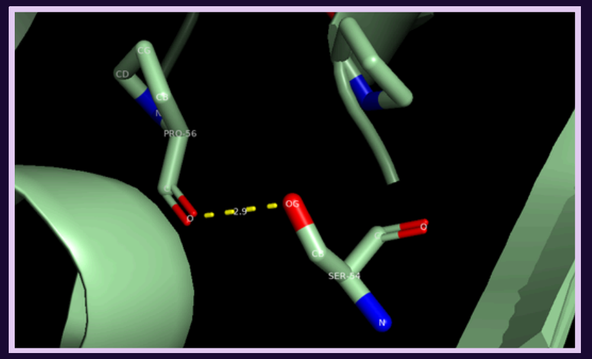
Pymol generated

Interpentamer interactions

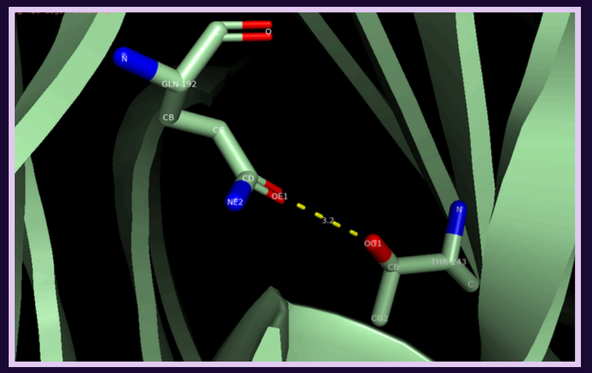
- The interfaces between the pentamers of the viral capsid are crucial for infection.
- The interactions determine:

1. Capsid assembly
2. Virion assembly
3. Virion stability
4. Virion infectivity

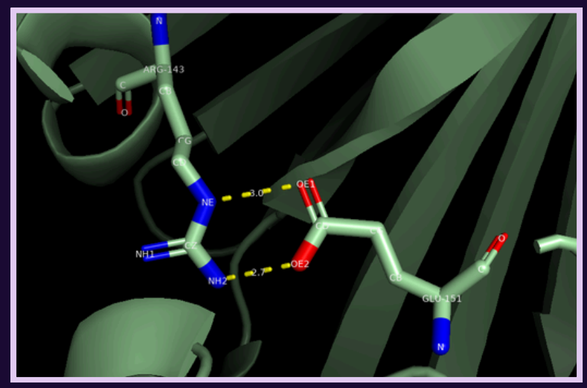
- The HRV interpentamer interface is atypical → infectivity-related residues densely distributed.
- The majority of residues involved in interactions do so through their side chains.
- The interface is mostly polar, with a high concentration of positively charged residues



PRO 56 (VP2) - SER 54 (VP2)



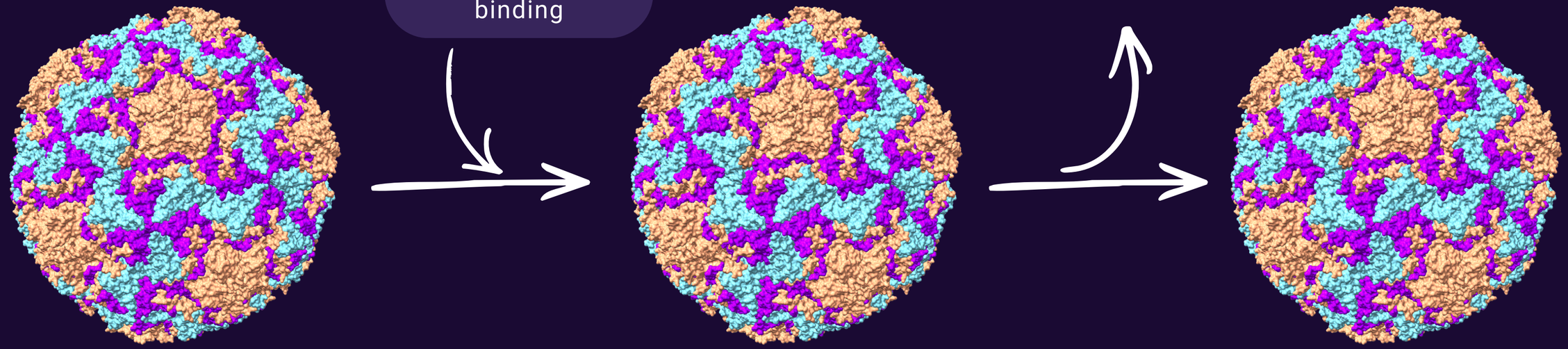
GLN 192 (VP3) - THR 243 (VP2)



ARG 143 (VP3) - GLU 151 (VP2)

Pymol generated

Viral infection



Mature capsid

VP1+VP2+VP3+VP4+RNA

Conformational changes

- Release of VP4 from the capsid
- Exposes VP1 N-ter segments

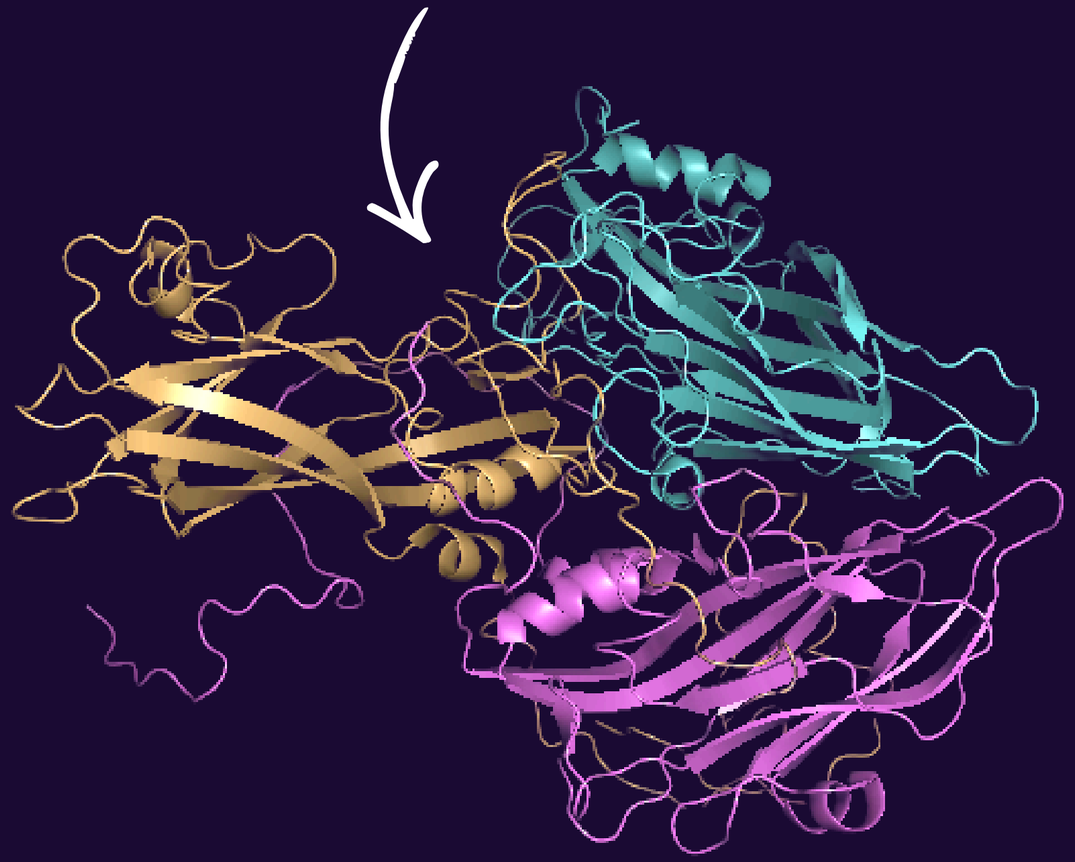
Empty capsid

VP1+VP2+VP3+VP4

VP1 VP2 VP3

Chimera generated

Canyon



A ~20-Å-deep canyon is located at the junction of VP1, VP2 and VP3

Forming the north rim

Forming the south rim

The canyon surrounds each of the 12 icosahedral 5-fold vertices

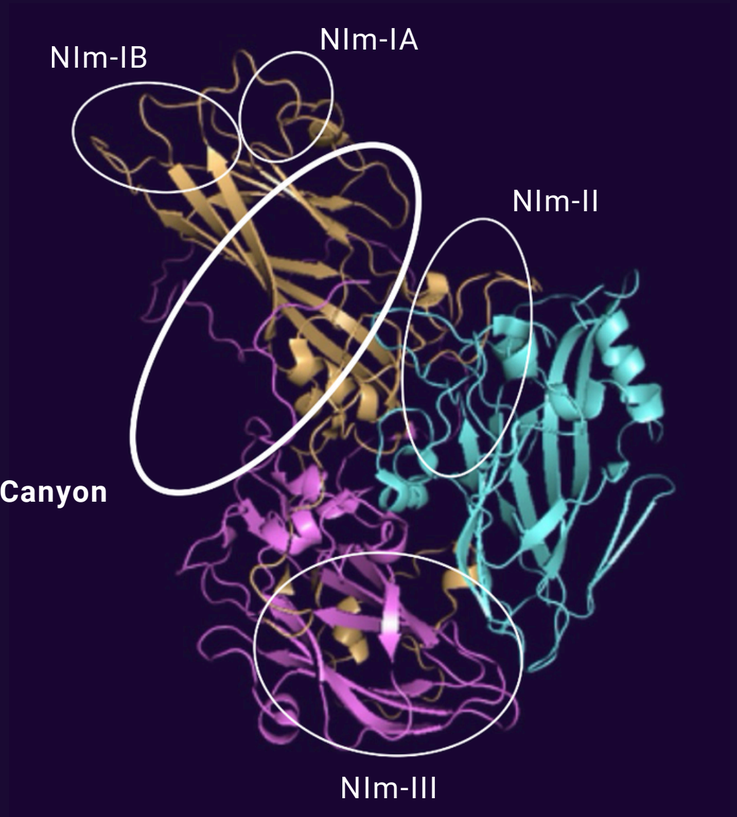
The canyon regions of HRV14 contain the binding site for the cellular receptor **ICAM-1**

VP1 VP2 VP3

Pymol generated

Canyon

Four major neutralizing immunogenic sites, NIm-IA, NIm-IB, NIm-II, and NIm-III, were identified by studies of neutralization escape mutants with monoclonal antibodies and then mapped to four protruding regions on the viral surface



Adapted from: Enterovirus Antibodies: Friends and Foes

- **NIm-IA** → Located on the VP1 protein
- **NIm-IB** → Located on the VP1 protein

} Northern rim of the canyon

- **NIm-II** → Found on the VP2 protein
- **NIm-III** → Located on the VP3 protein

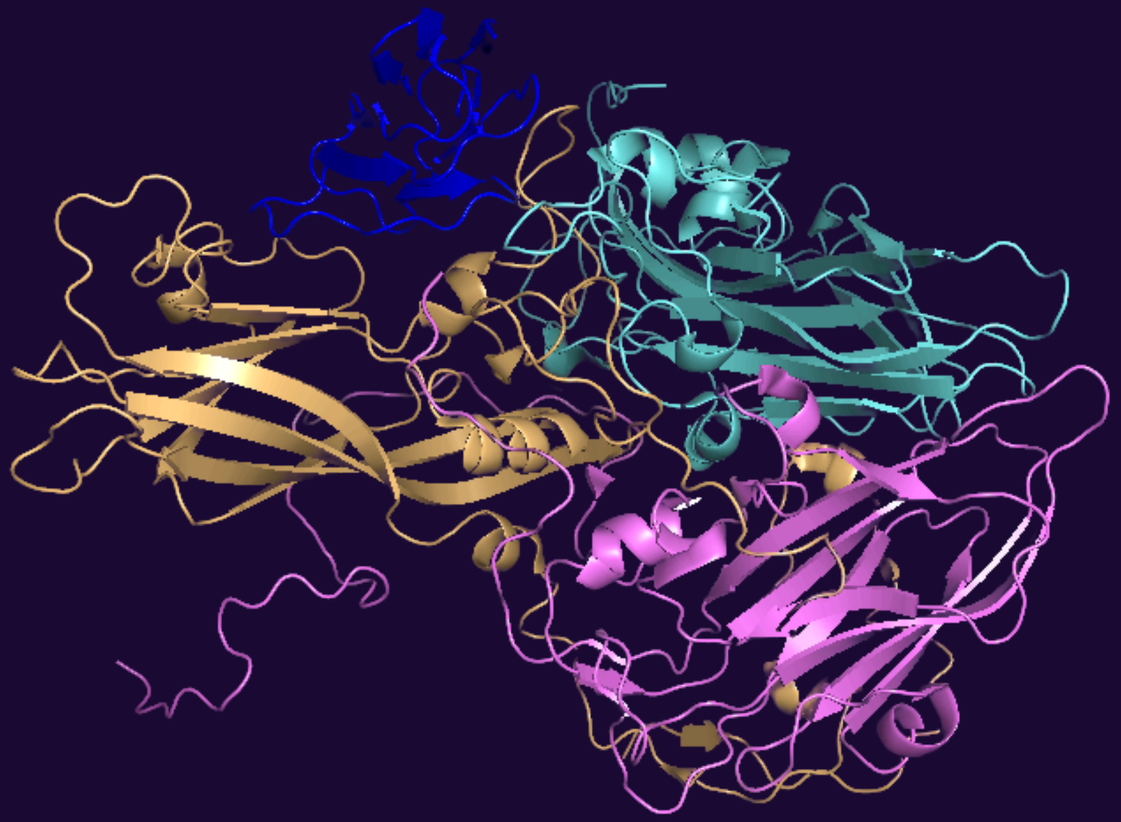
} Southern rim of the canyon

VP1 VP2 VP3

Pymol generated

Canyon

Interactions with ICAM-1



VP1
VP2
VP3
ICAM-1

7BG7
Resolution: 2.40 Å

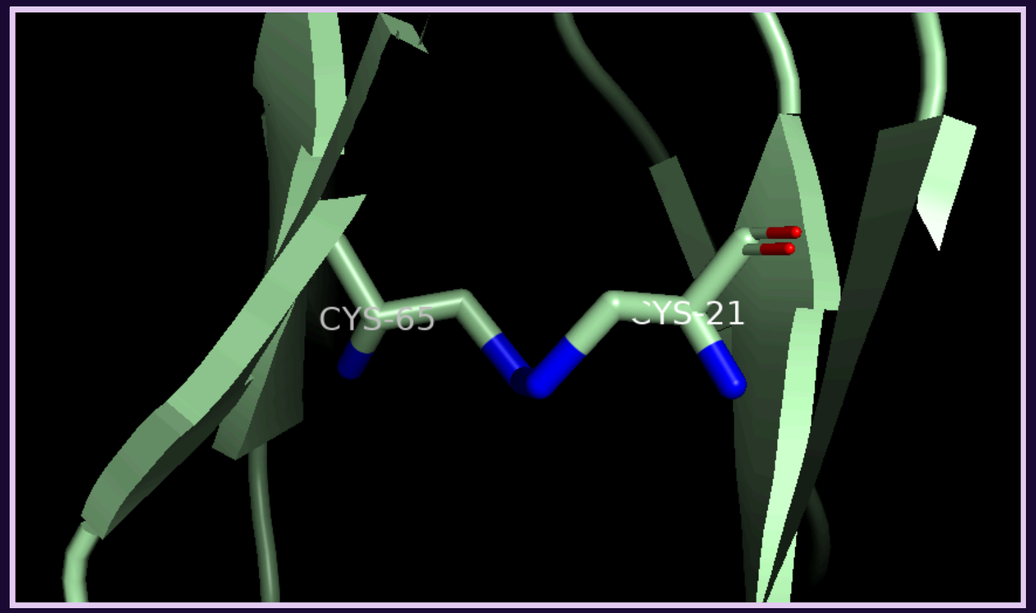
Pymol generated

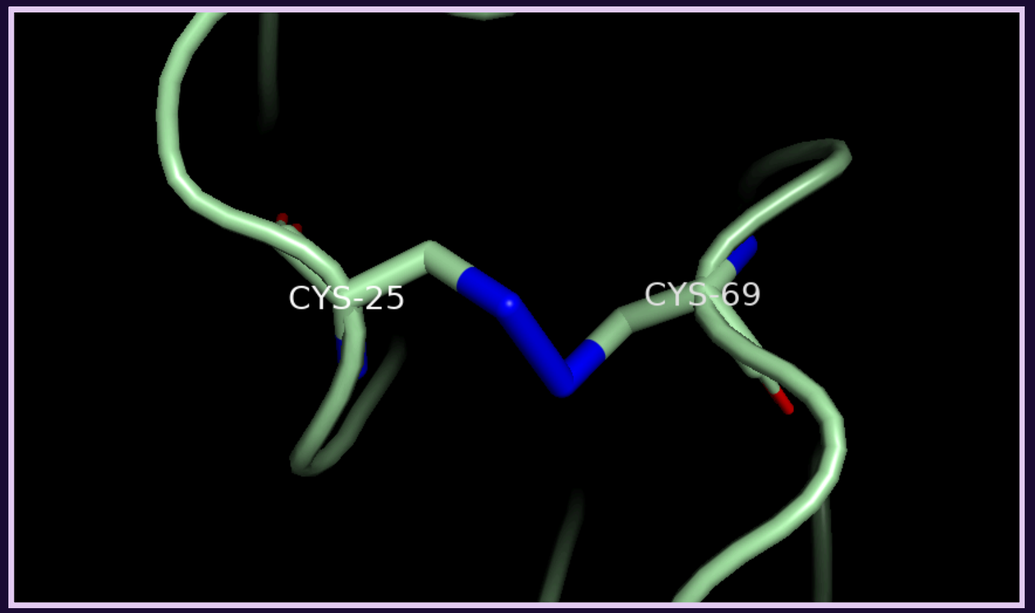
Canyon

Interactions within ICAM-1

Disulfide bonds

The structures of domain 1 of ICAM-1 determined to date contain the disulfide bonds Cys21 and Cys65 and Cys25 and Cys69



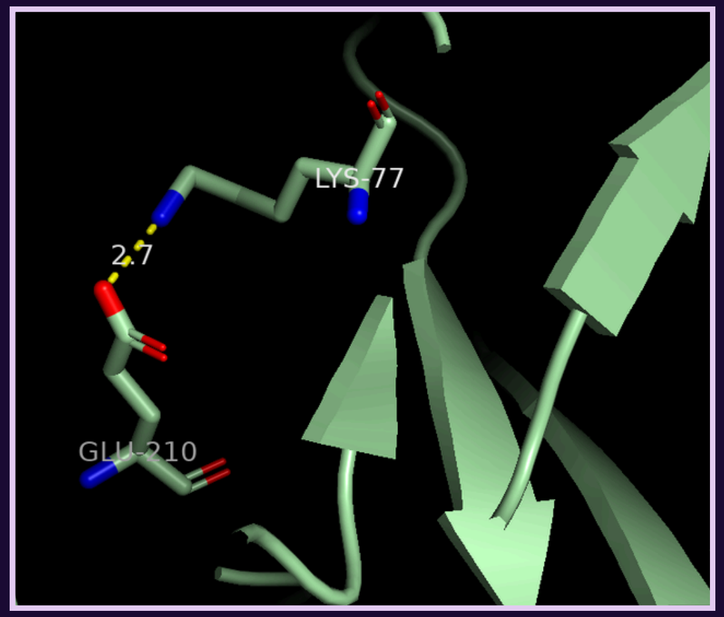


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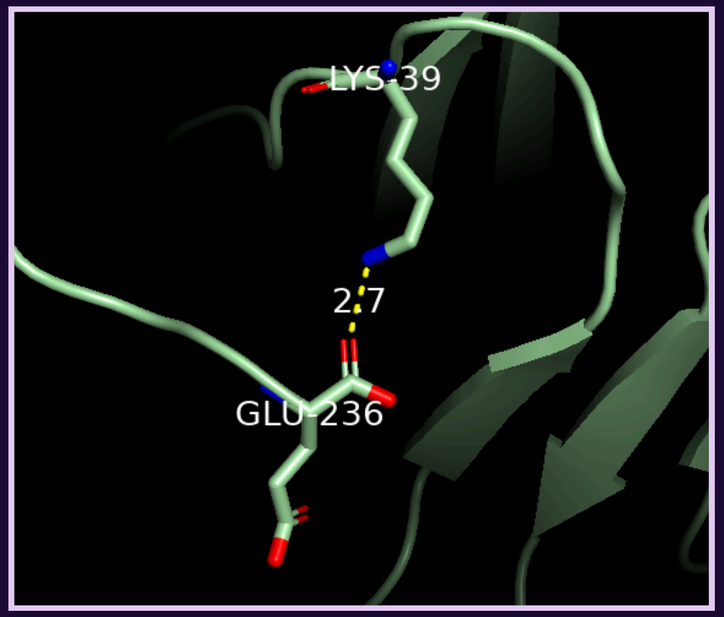
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Interactions with ICAM-1

Salt bridges



Salt bridge interaction between Lys77 of ICAM-1 and Glu210 of the capsid protein VP1



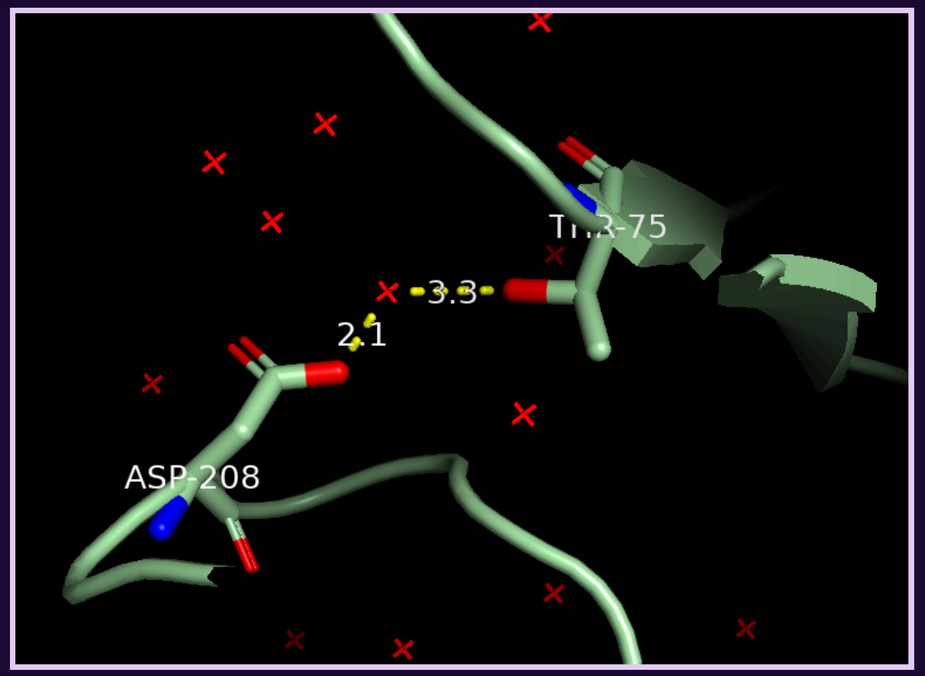
Salt bridge interaction between Lys39 of ICAM-1 and Glu236 of the capsid protein VP3

Pymol generated

Canyon

Interactions with ICAM-1

Hydrogen bonds



The residue Thr75 of ICAM-1interacts with a water molecule that forms a hydrogen bond with Asp208 of protein VP1

The interaction between the residues Thr75 and Asp208 is characterized by distances of 2.1 Å and 3.3 Å, respectively.

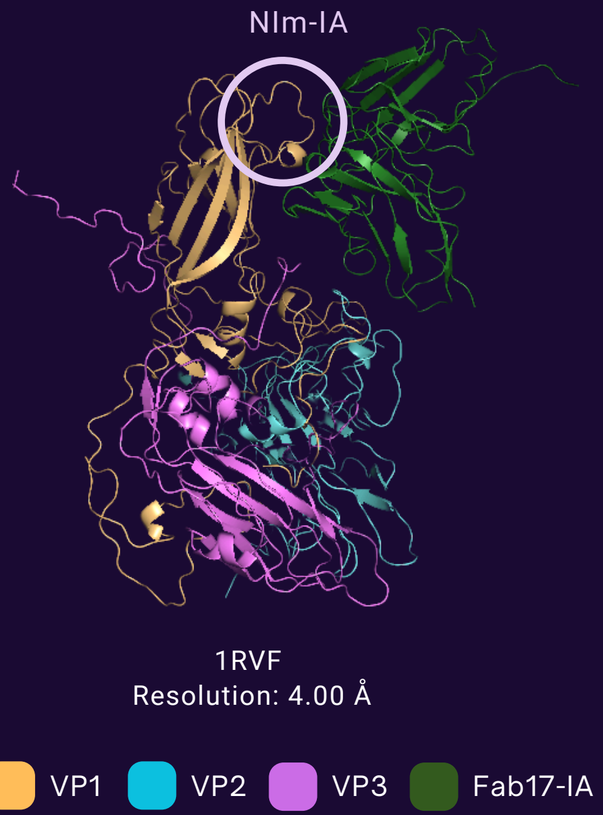
Pymol generated

Canyon

Interactions with Fab17-IA

The monoclonal antibody 17-IA binds to the **NIm-IA** site on HRV-14

The variable domains of Fab17-IA make contact with both the north and south walls of the canyon

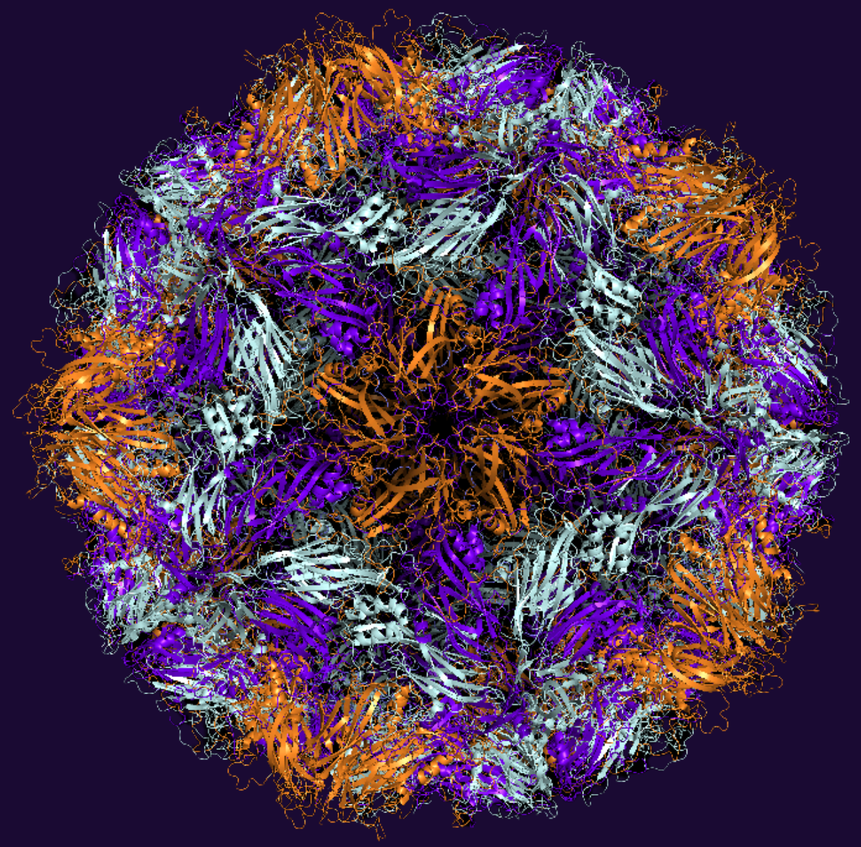


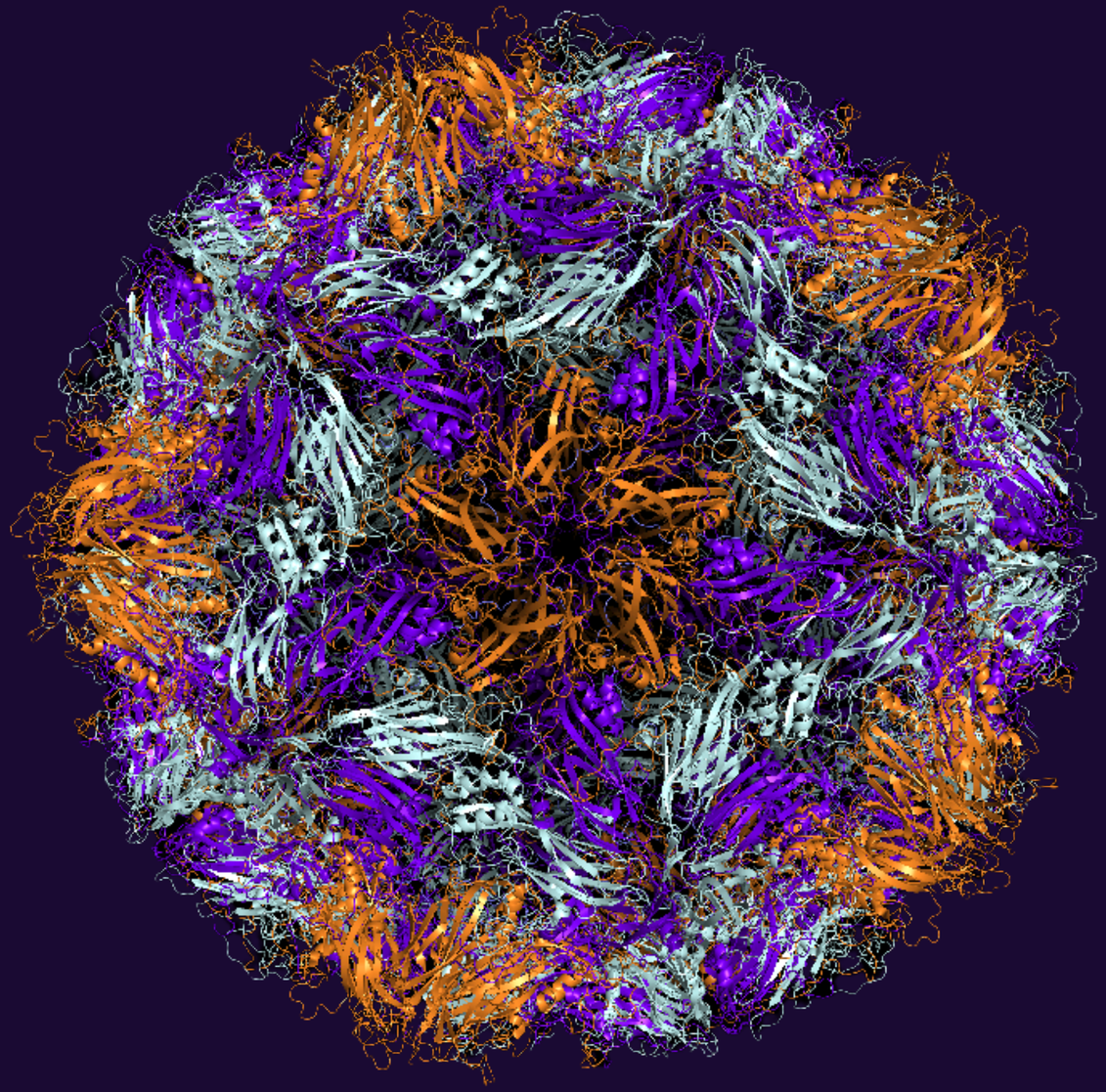
- Asp54 H and Asp56 H in the CDR2 loop of Fab17 interdigitate between Arg1094 and Lys1097 in HRV-14
- Four residues (His1078, Val1079, Thr1080, and Asp1101) at the bottom of the north side of the canyon interact with the bound Fab17
- Arg91 L forms salt bridges with the two natural escape mutation sites (Glu1095 and Asp1091)
- A cluster of lysines on HRV-14 (K1236, K1097, K1085) interacts with acidic residues (Asp45 H and Asp54 H) in the CDR2 loop of Fab17
- Charged residues at the capsid-Fab17 interface are key determinants of binding

Pymol generated

Conclusions

- The three proteins have evolved differently, providing picornaviruses with surface characteristics that enable them to resist immune pressure.
- The extent the similarity among the three dimensional structures of all of the small RNA icosahedral virus coat proteins suggests that they are derived from a common ancestor
- Rhinovirus VP1, VP2 and VP3 proteins share a common jelly-roll fold, with notable differences in certain regions.
- The structural variability among the proteins could have functional implications in their interaction with cellular receptors and in the formation of the capsid.





THANK YOU FOR YOUR ATTENTION

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PEM questions

1. How is the viral RNA released into the host cell after the rhinovirus binds to ICAM-1?

a) Through endocytosis followed by RNA synthesis in the nucleus
b) By the disintegration of the capsid, releasing the RNA directly
c) Through fusion with the host's membrane, leading to direct RNA entry
d) By the externalization of VP1, creating a pore in the cell membrane
e) By cleavage of the viral RNA at the 3' end, enabling release

2. Which receptor does rhinovirus primarily bind to during infection?

a) LDLR (low-density lipoprotein receptor)
b) CD4 receptor
c) ICAM-1 (Intercellular Adhesion Molecule-1)
d) MHC Class I
e) Integrin receptor

3. What role does the canyon region play in rhinovirus infection?

a) It assists in the uncoating of the virus
b) It binds to cellular receptors, facilitating viral entry
c) It prevents RNA replication
d) It maintains the stability of the capsid during transmission
e) It stabilizes the viral genome for long-term persistence

4. Which of the following describes the icosahedral symmetry of the rhinovirus capsid?

a) The virus is composed of 12 vertices with 3-fold symmetry
b) There are 60 identical protomers forming the viral surface
c) VP4 forms the external structure of the capsid
d) The capsid consists of a hexagonal arrangement of proteins
f) The capsid is enveloped with lipid bilayers

PEM questions

5. Which of the following statements about the antiviral compound R 61837 is true?

a) It interacts with the hydrophobic pocket, preventing viral RNA release.
b) It forms a pi-pi interaction with tyrosine 128.
c) It induces conformational changes in the virus, making the protein coat more flexible.
d) It disrupts the formation of pentamers in the viral capsid.
e) All the above are correct.

6. What is the role of serine 10 in VP2 during the maturation of the virus?

a) It helps form a hydrogen bond with pyridazine in antiviral compounds.
b) It plays a crucial role in the autoproteolytic cleavage of VP0.
c) It stabilizes the β-cylinder structure of VP3.
d) It anchors the viral RNA to the capsid during uncoating.
e) It is involved in receptor binding on the viral surface.

7. What structural feature of the viral capsid is primarily involved in the assembly of protomers into pentamers?

a) Hydrophobic interactions between the β-cylinder and VP1
b) The β-cylinder structure formed by VP3
c) The jelly roll structure found in VP1, VP2, and VP3
d) Salt bridges between adjacent pentamers
e) Disulfide bonds between VP3 molecules

8. Regarding the VP1, VP2, and VP3 proteins of rhinovirus, which of the following statements is correct?

a) VP3 is the most structurally different protein
b) They are homologous proteins with more than 60% identity between them
c) They share a common fold but are very different at the sequence level
d) VP2 forms a canyon involved in receptor binding
e) All of the answers are incorrect

PEM questions

9. Which type of interaction occurs more frequently within the protomer compared to interprotomer or interpentamer interactions?

a) Hydrophobic interactions, mainly between VP1 and VP3
b) Hydrophobic interactions, mainly between VP2 and VP4
c) Hydrophobic interactions, mainly between VP1 and VP4
d) Hydrogen bonds, most of which are water-mediated
e) Salt bridges

10. Regarding the structural alignment of the three capsid proteins of Rhinovirus 14, which of the following statements is correct?

a) The β-barrel strands have the best superposition
b) The loops between the β-strands are the most variable regions among VP proteins
c) Two of the above are correct
d) The C-terminal tail of VP1 fits surprisingly well with the knob loop of VP3
e) All of the answers are correct