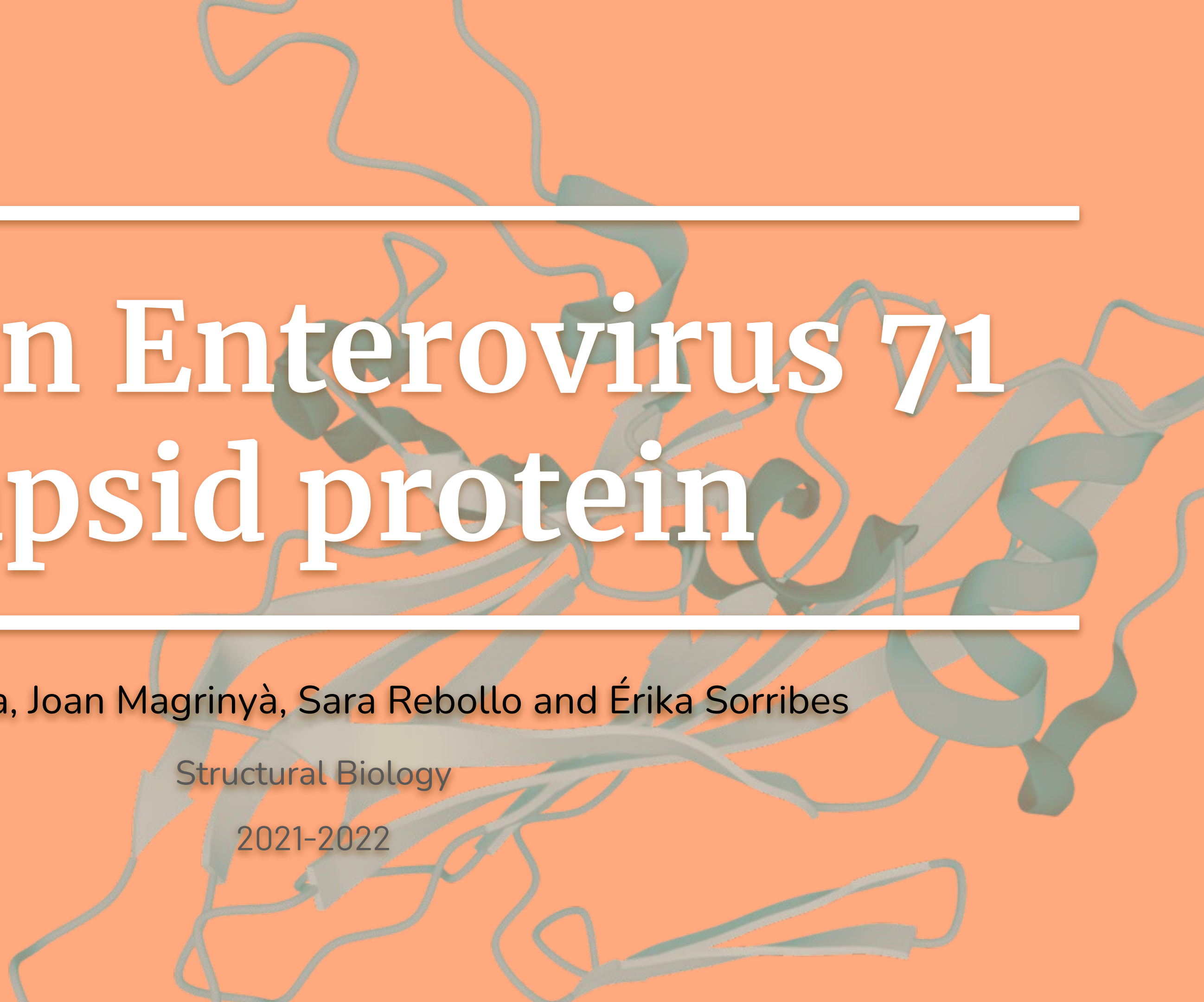

Human Enterovirus 71 capsid protein

Carla Aranda, Joan Magrinyà, Sara Rebollo and Érika Sorribes

Structural Biology

2021-2022

Index

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INTRODUCTION

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PHYLOGENETIC ANALYSIS

Conservation analysis among picornavirus

Human Enterovirus 71 and phylogeny

Picornaviridae family

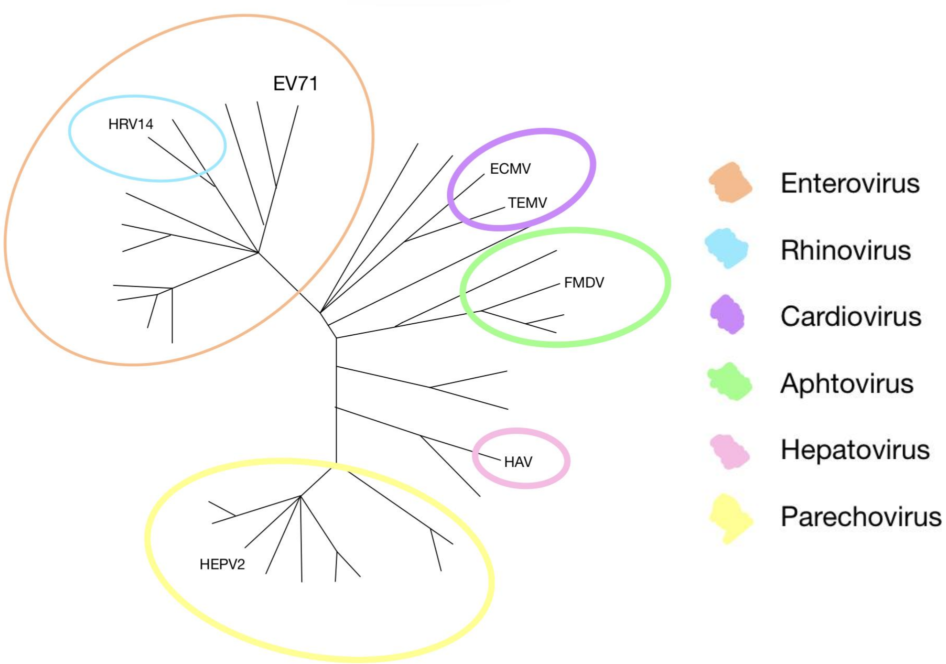
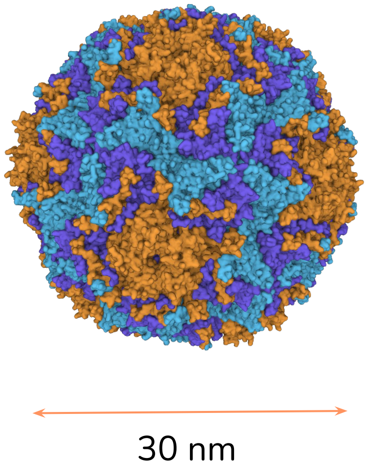


Fig. 1: Phylogenetic tree of *Picornaviridae* family.
Adapted from Benschop, et al. 2006.

EV71



Non-enveloped
ssRNA+ virus

VP1
VP2
VP3

Clinical manifestations

HFMD

Hand, foot and mouth disease

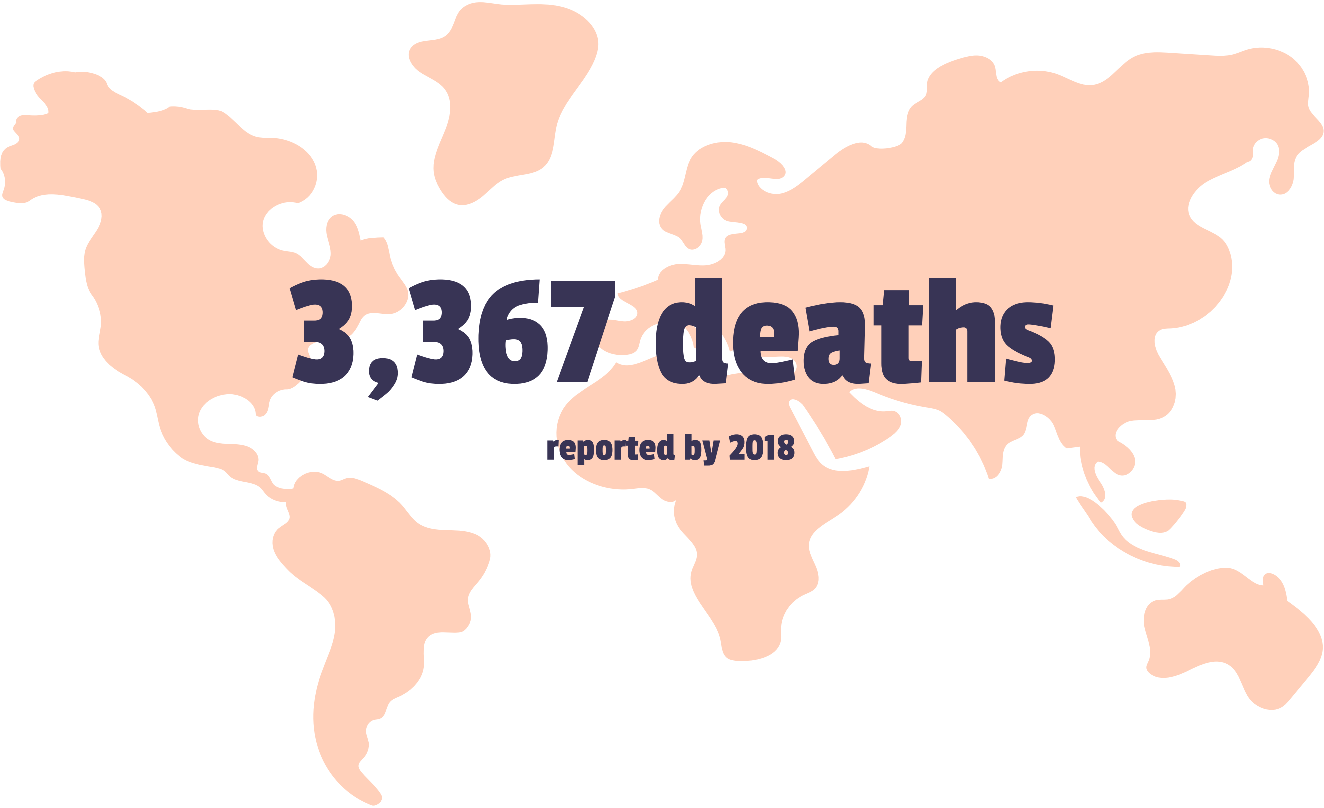
NEUROLOGICAL MANIFESTATIONS

Aseptic meningitis to acute flaccid paralysis and brainstem encephalitis

CARDIOPULMONARY FAILURE

Oedema and shock

Epidemiology



3,367 deaths

reported by 2018

Life cycle

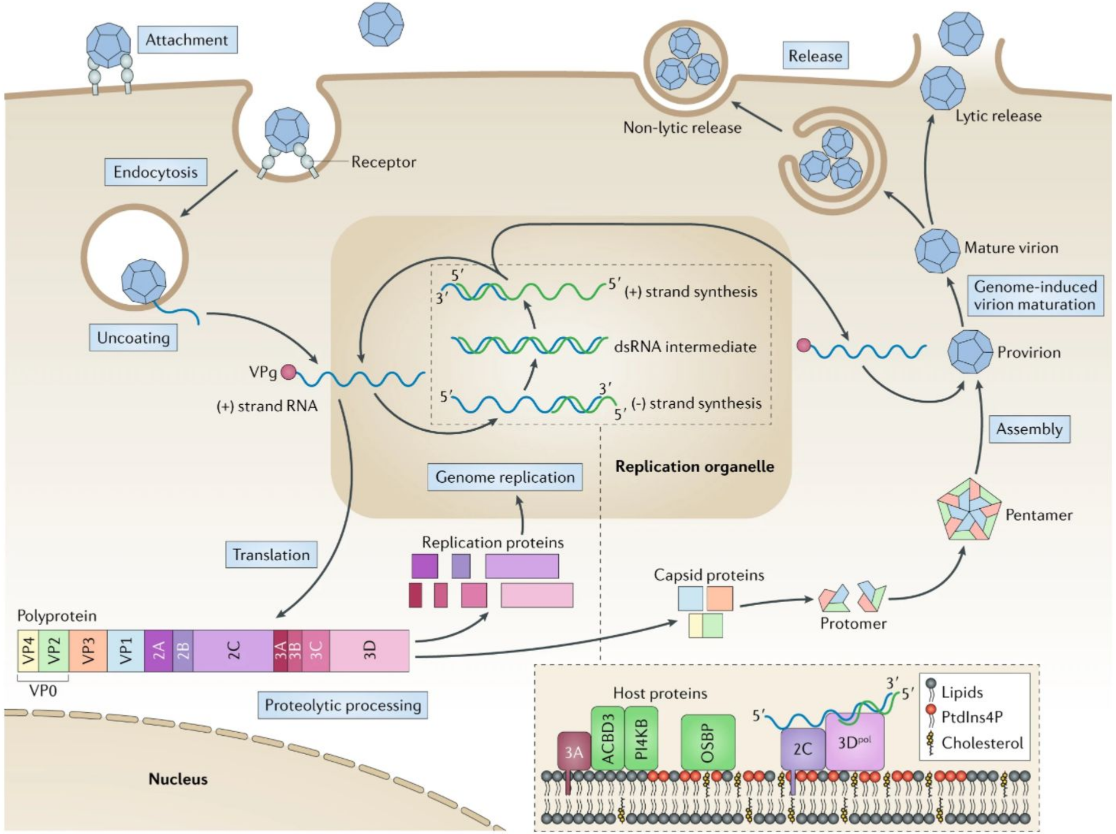


Fig. 2: HEV71 life cycle . Baggen J, et al. 2018

Genome

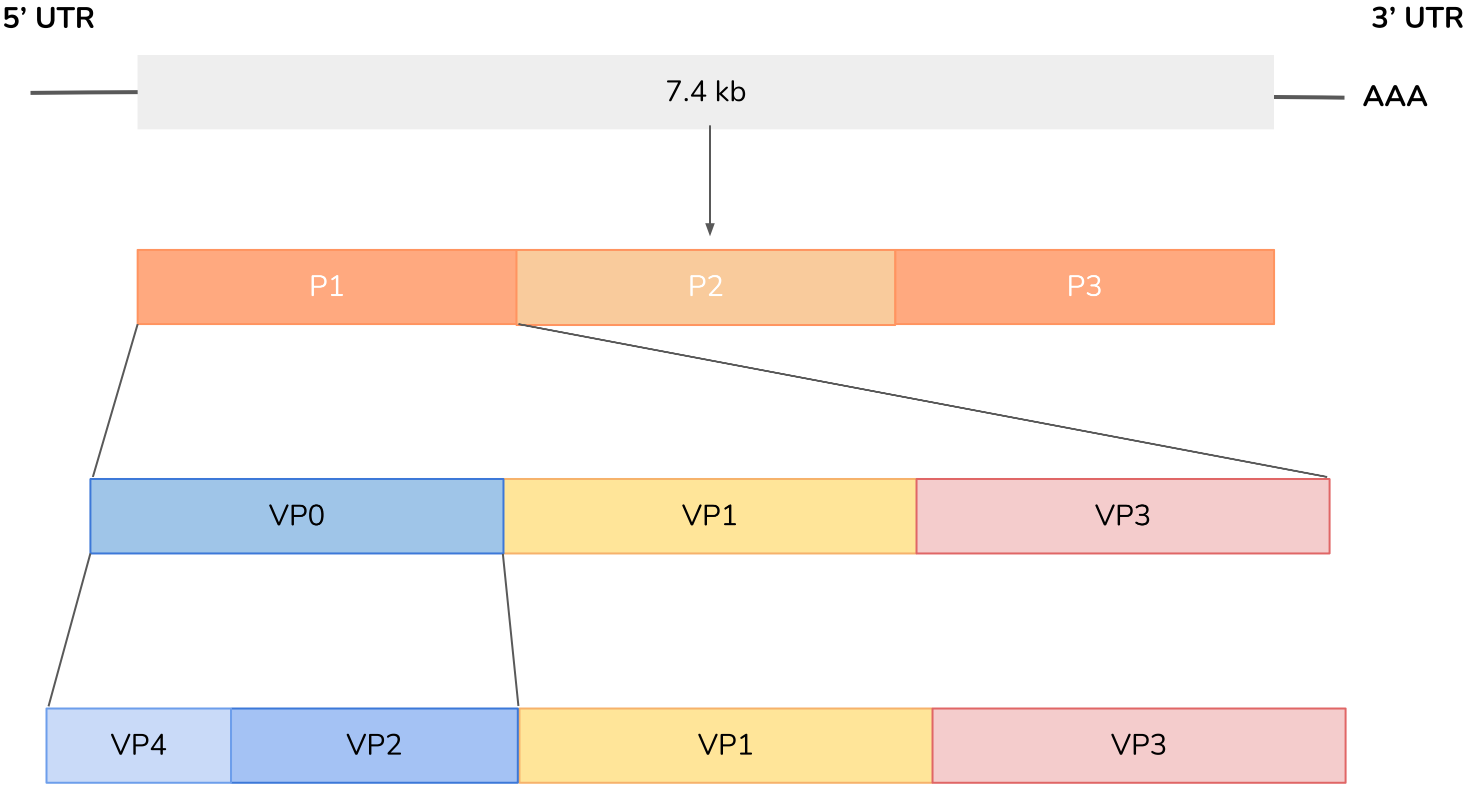
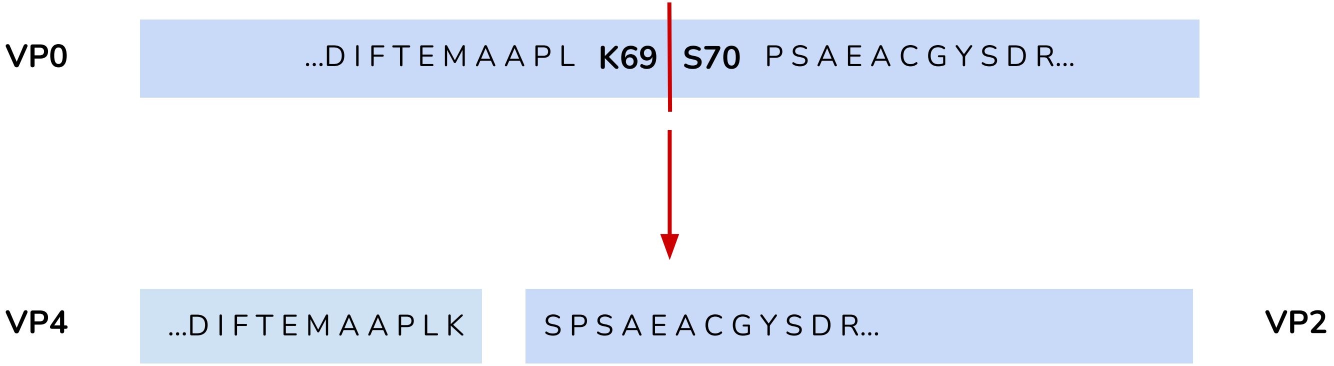


Fig. 3: HEV17 genome. Adapted from Cao J, et al. 2019.

Cleavage of VP0



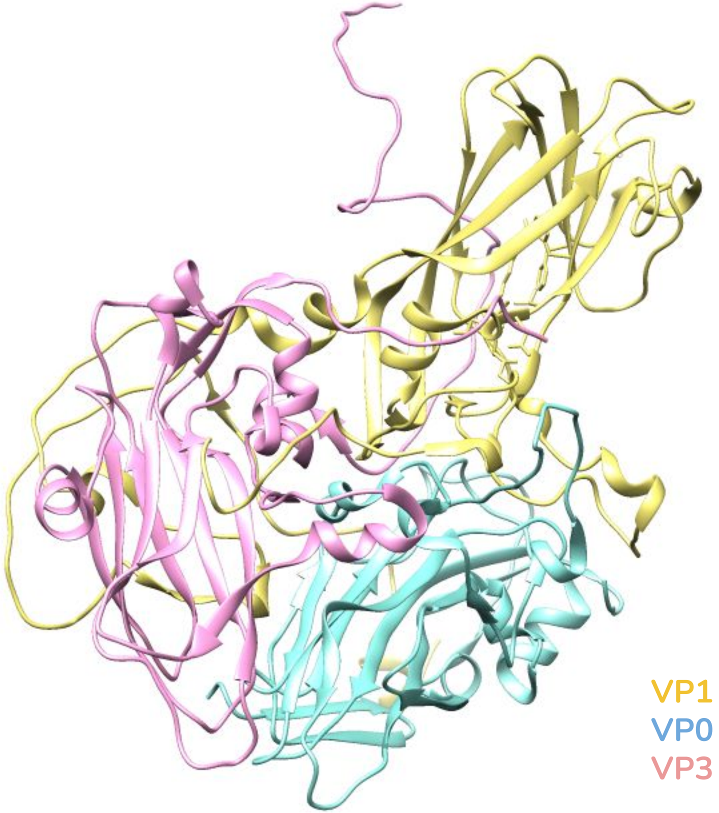
VP0 ...DIFTEMAAPL **K69** **S70** PSAEACGYSDR...

VP4 ...DIFTEMAAPLK

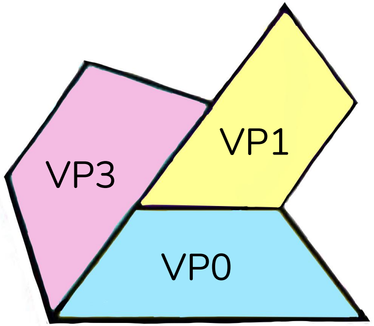
VP2 SPSAEACGYSDR...

Fig. 4: VP0 cleavage. Adapted from Cao J, et al. 2019.

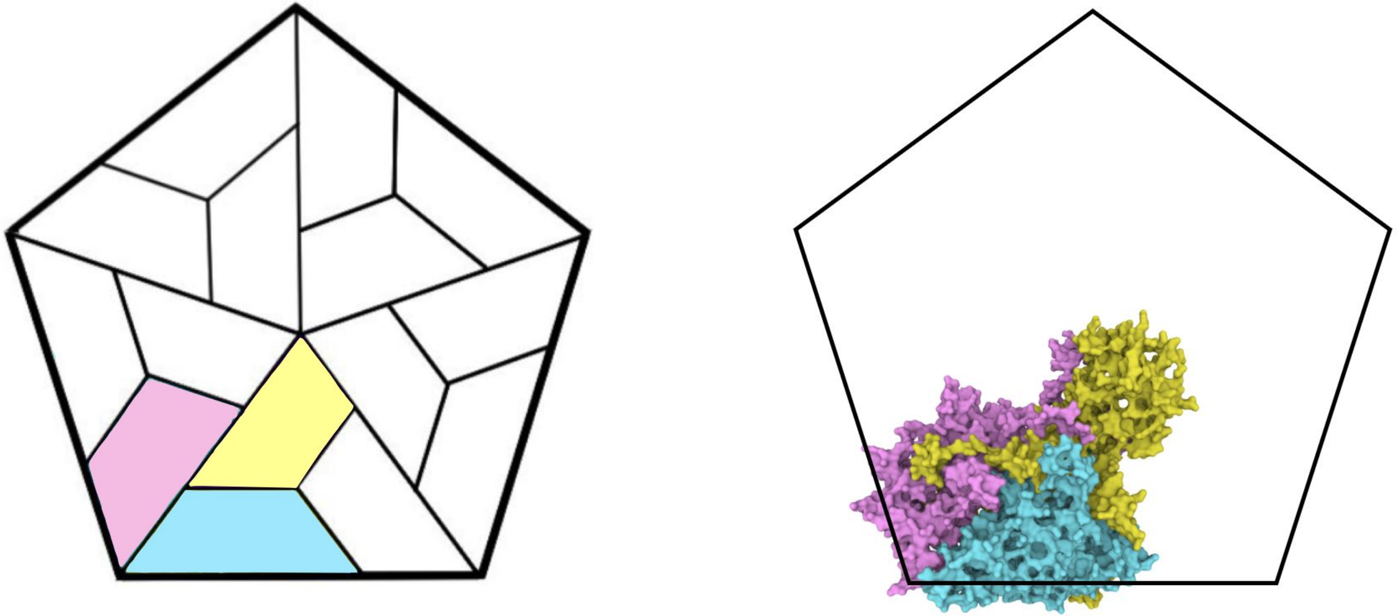
Capsid assembly



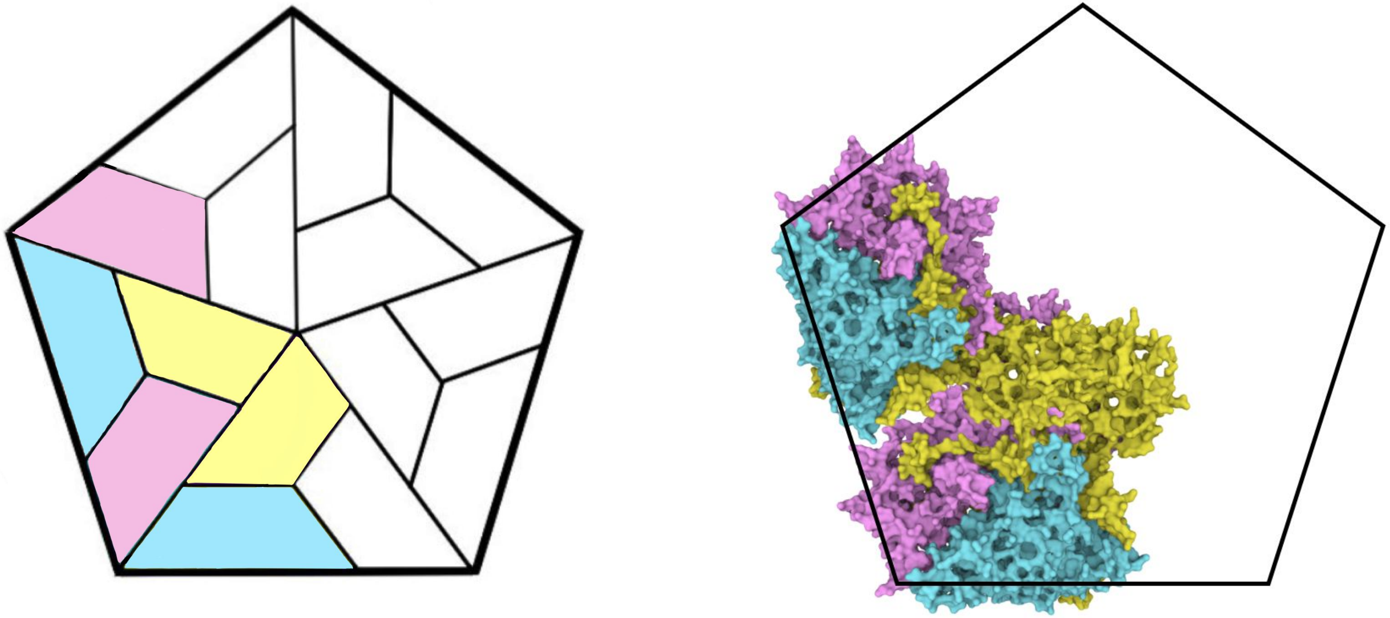
VP0 + VP1 + VP3 = **protomer**



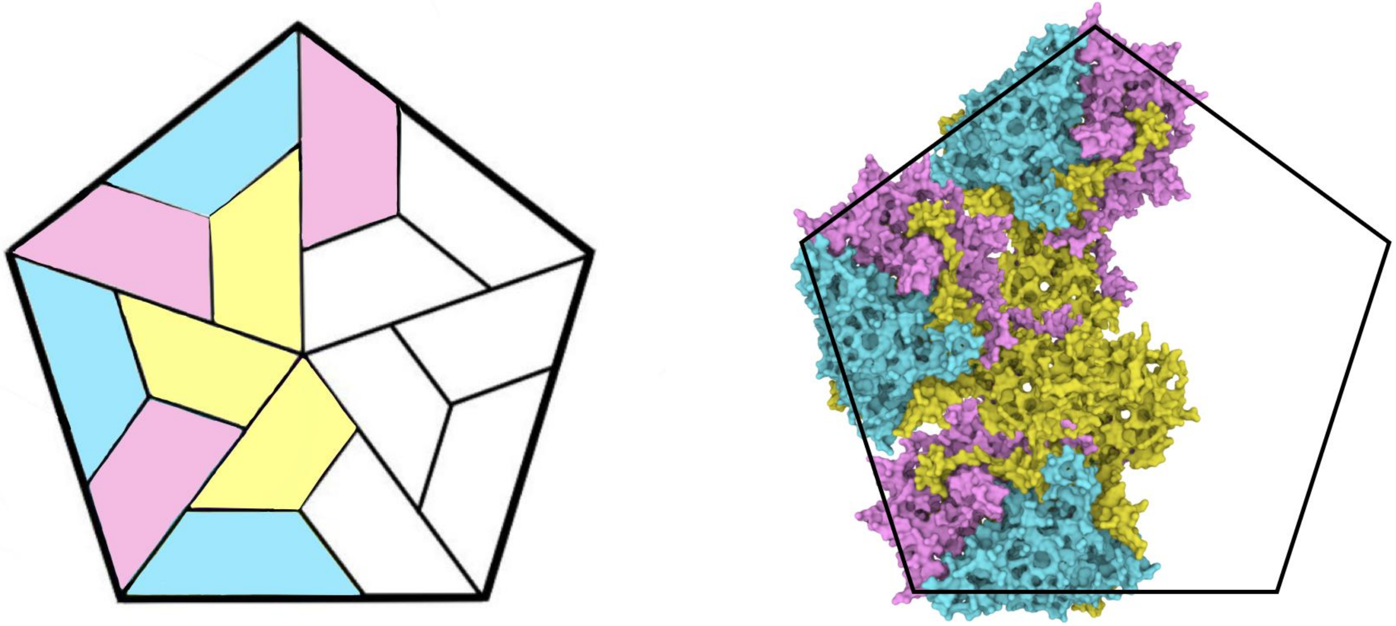
Capsid assembly



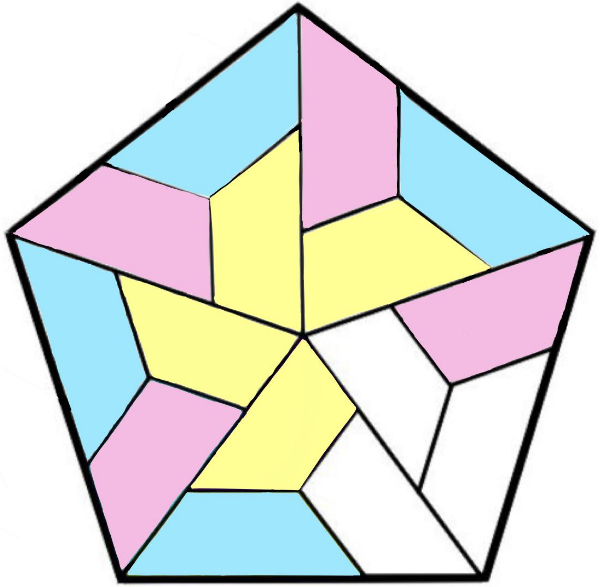
Capsid assembly

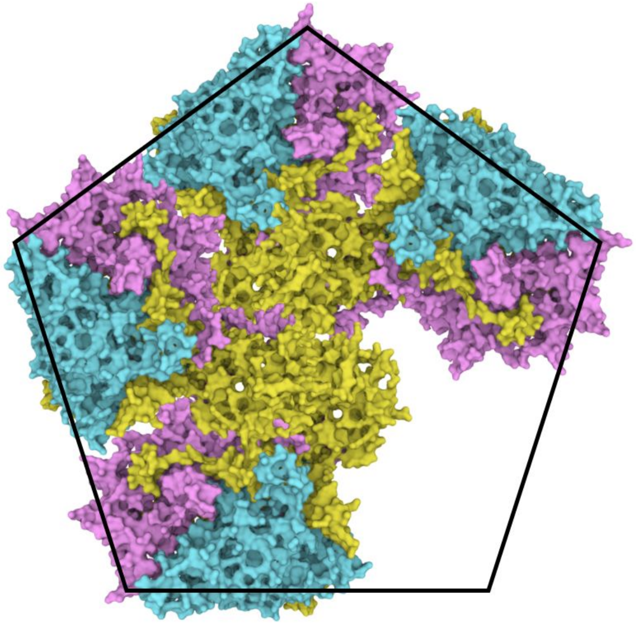


Capsid assembly



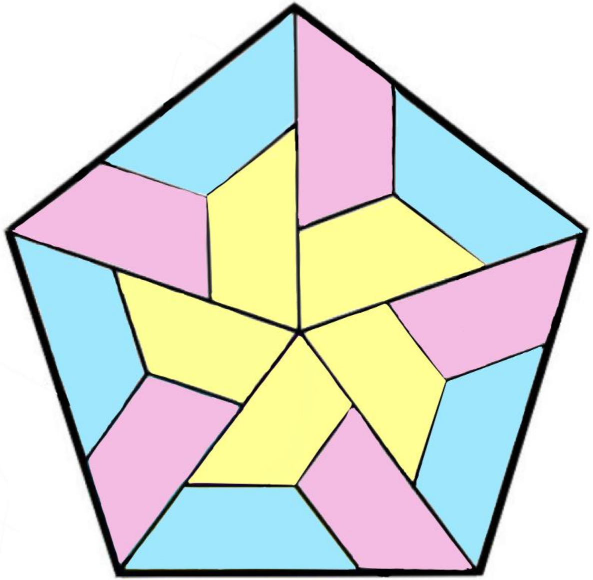
Capsid assembly

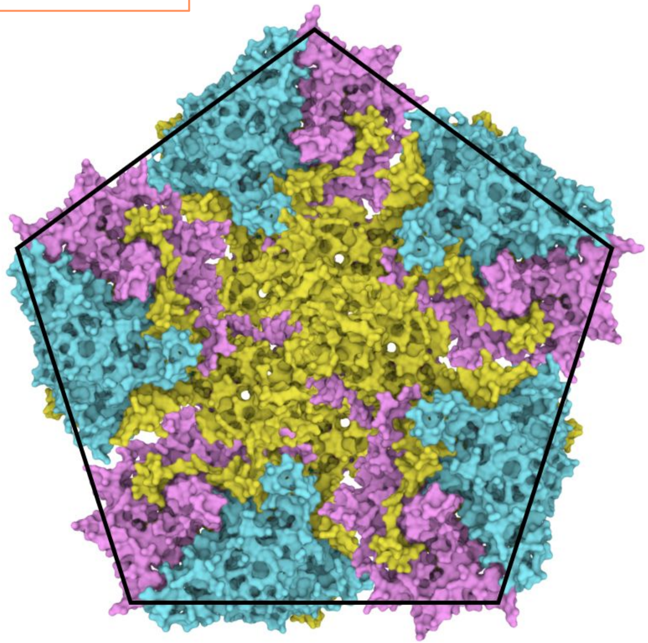




Capsid assembly

5 protomers form a capsomer





Capsid assembly and viral phases

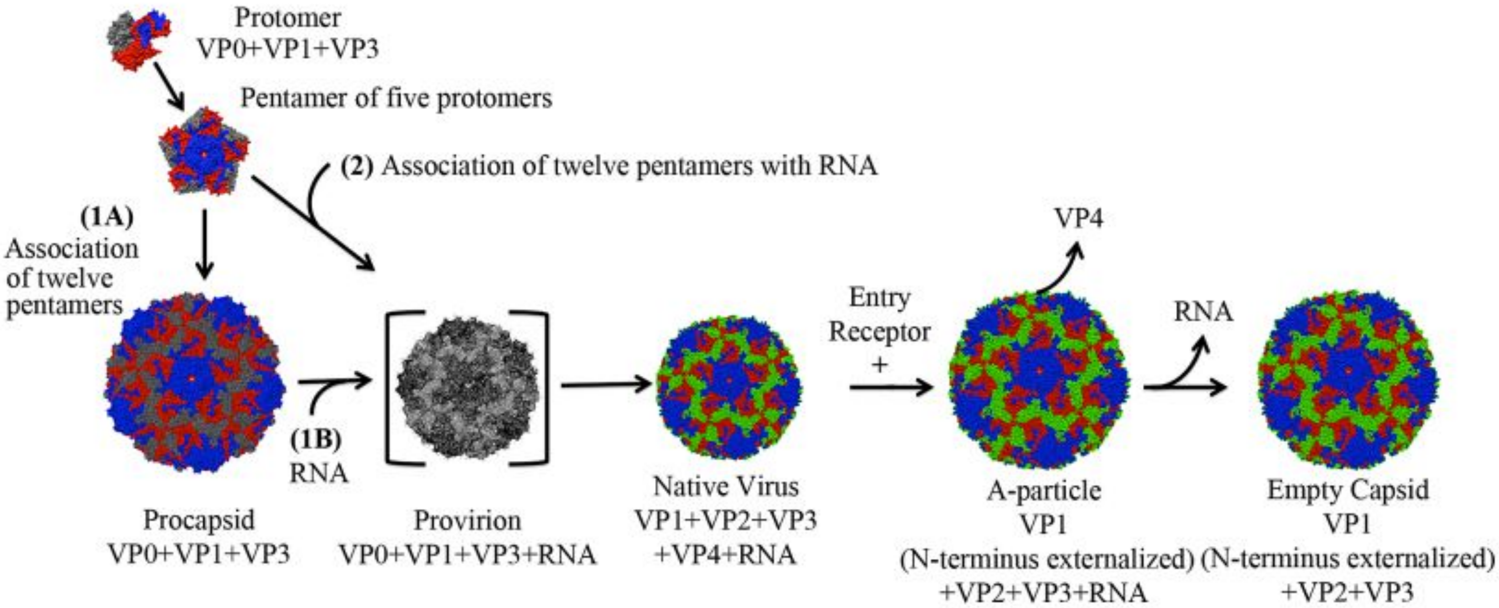
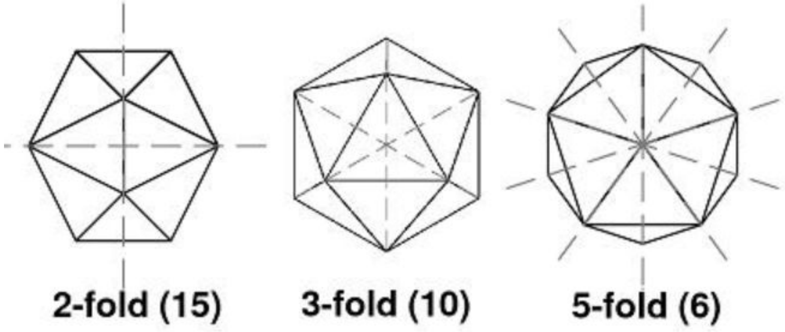


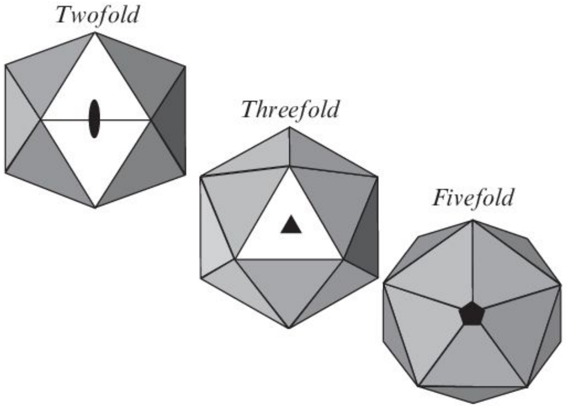
Fig. 5: HEV71 viral phases. Shinger KL, et al. 2013.

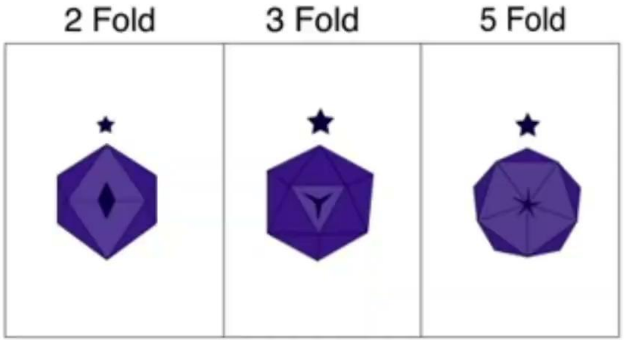
Axis of symmetry



Rotational symmetry is based on 2-3-5 Fold Symmetry:

- Two-fold axis: through the centre of each edge
- Three-fold axis: through the centre of each face
- Five-fold axis: through the centre of each vertex





Triangulation Number

Triangulation number gives us an idea of how big the capsid is and how many subunits compose the total capsid

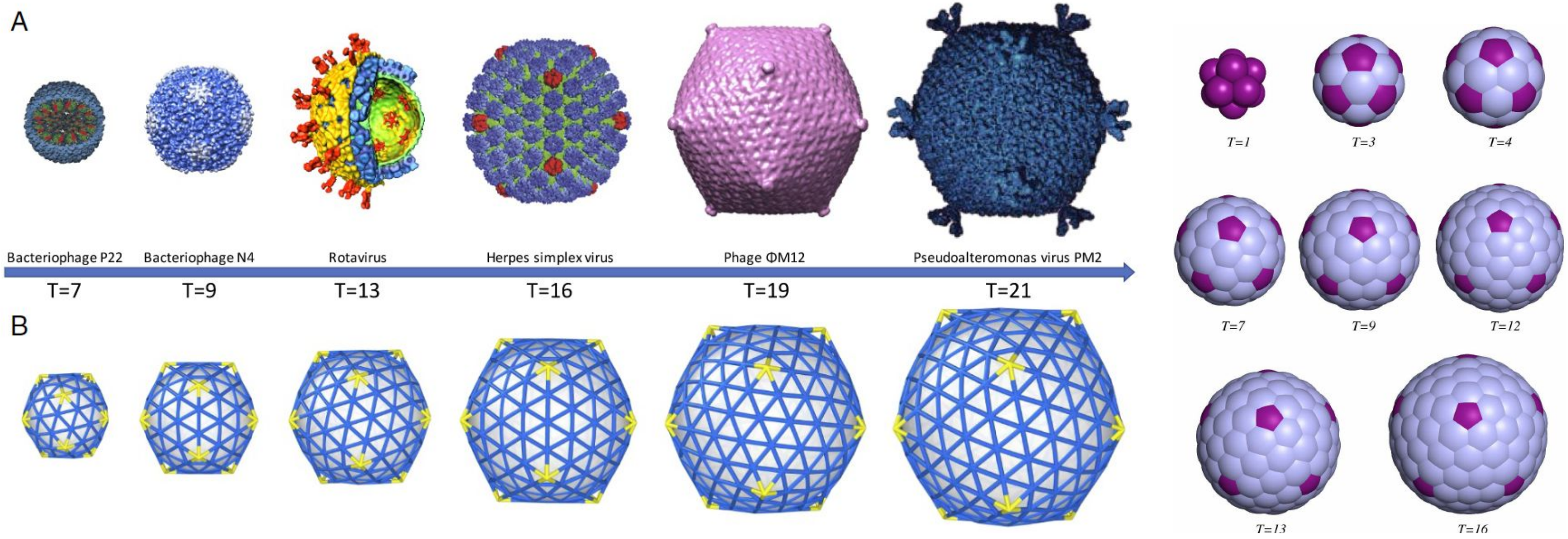


Fig. 6: Triangulation number. Cann, Alan J. et al. 2004.

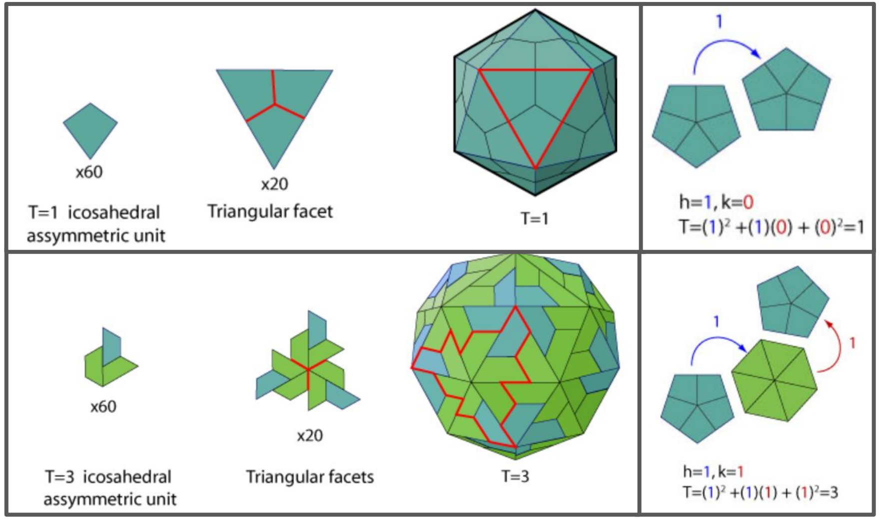
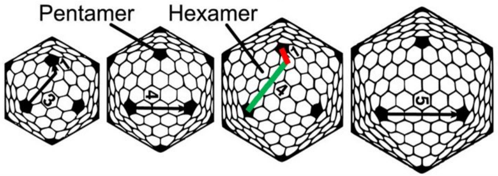


Fig. 7: Triangulation. Swiss institute of bioinformatics. 2015

$$T = h^2 + hk + k^2$$



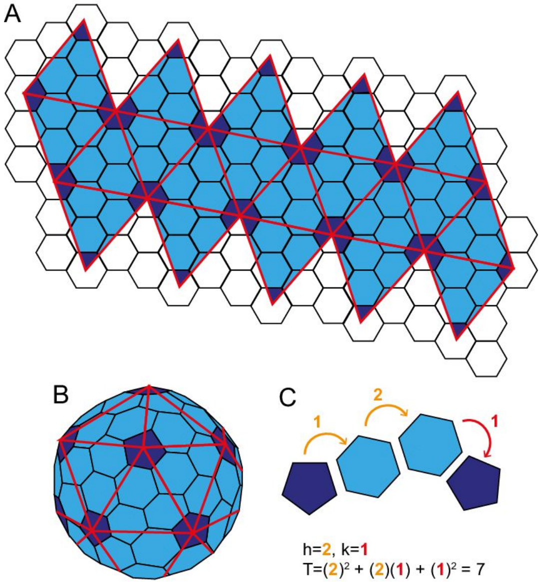


Fig. 8: Structural Studies of Viruses and Toxicological Studies. Seibert M. 2012

Quasi-equivalence and Pseudo T3

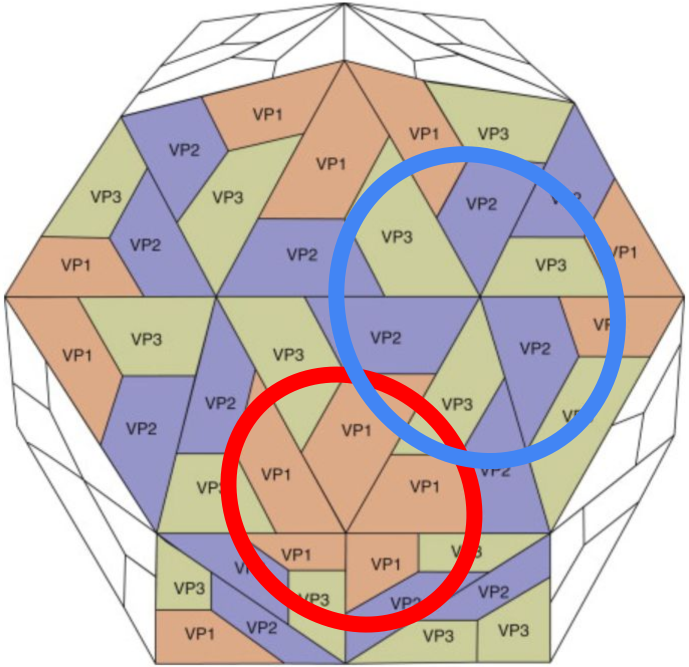


Fig. 9: Principles of Molecular Virology. Cann, Alan J. et al.

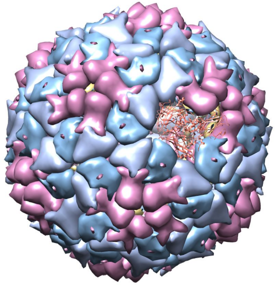
Equivalence:

- Each triangle is composed by 3 equal portions
- Each protein is the same environment

When T increases...

Quasi-equivalence:

- The proteins composing each triangle vary
- Positioning of each protein is not equivalent: proteins have different neighbours



SCOP

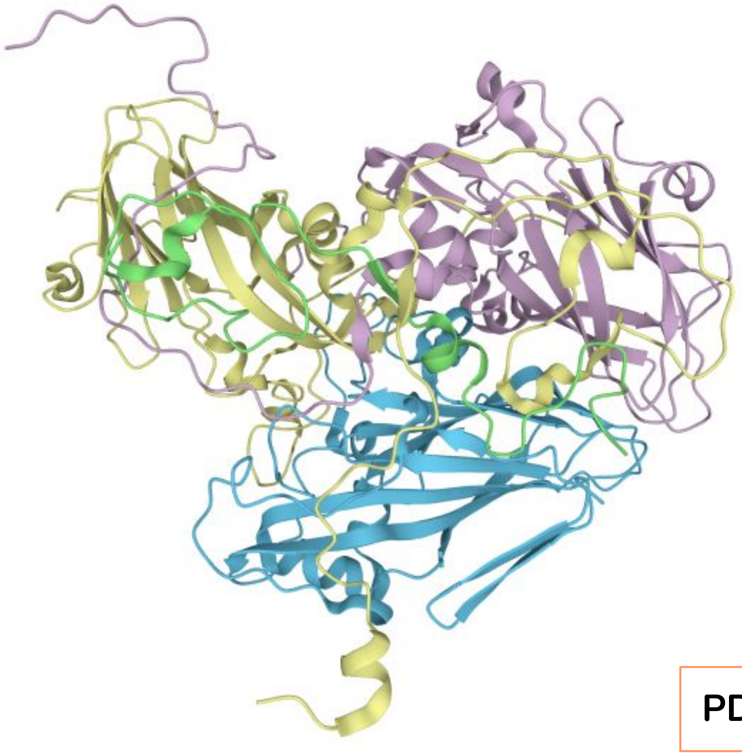
Class:
All beta proteins

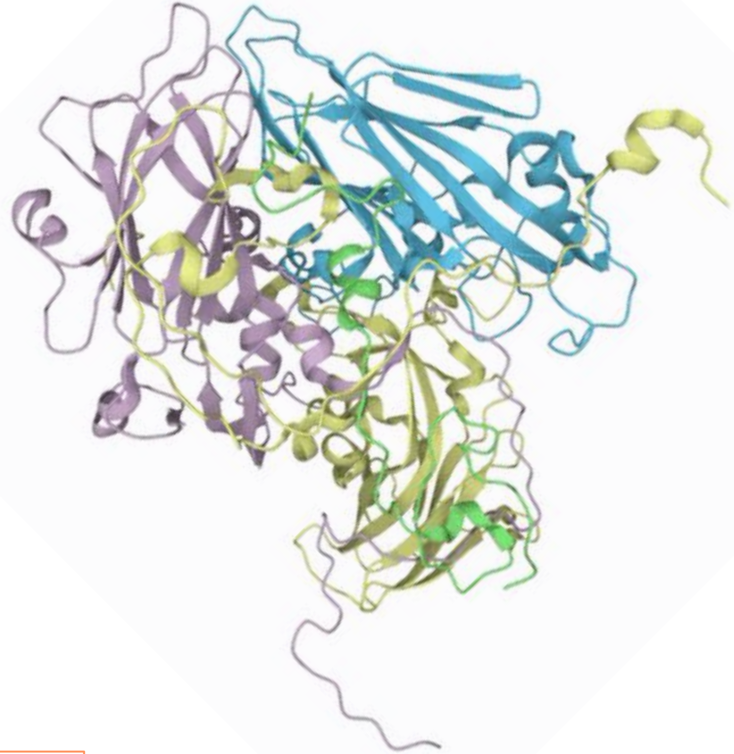
Fold:
Nucleoplasmin-like/VP (viral coat and capsid proteins)

Superfamily:
Positive stranded ssRNA viruses-like

Family:
Picornaviridae-like VP (VP1, VP2, VP3 and VP4)

Polyprotein 1: capsid protein

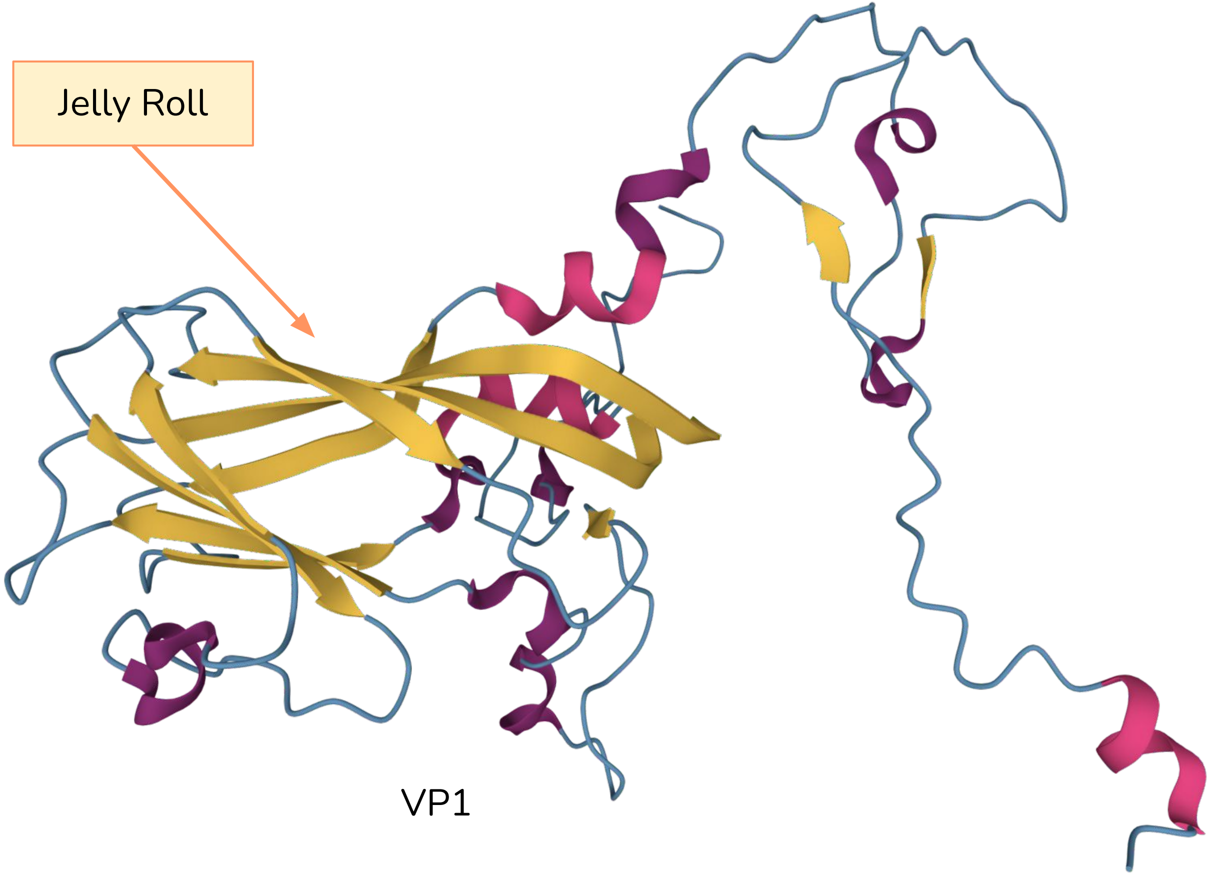




PDB entry: 3VBS

VP1
VP2
VP3
VP4

Asymmetric unit: VP1



VP1

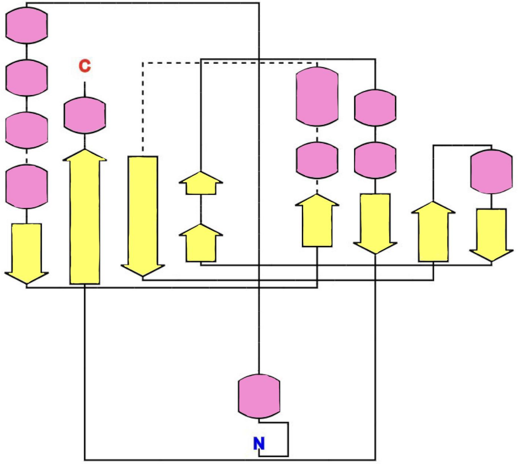


Fig. 10: Topological diagram of the secondary structures of VP1

Asymmetric unit: VP2

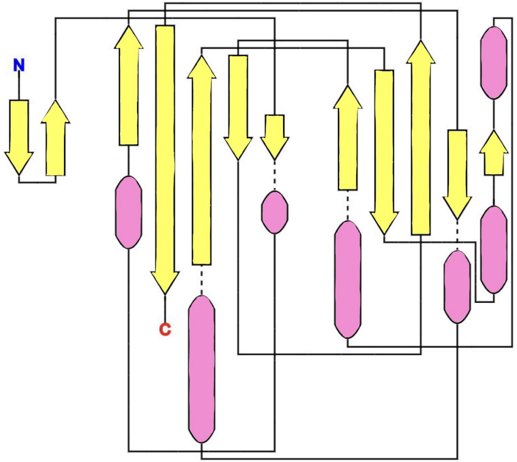
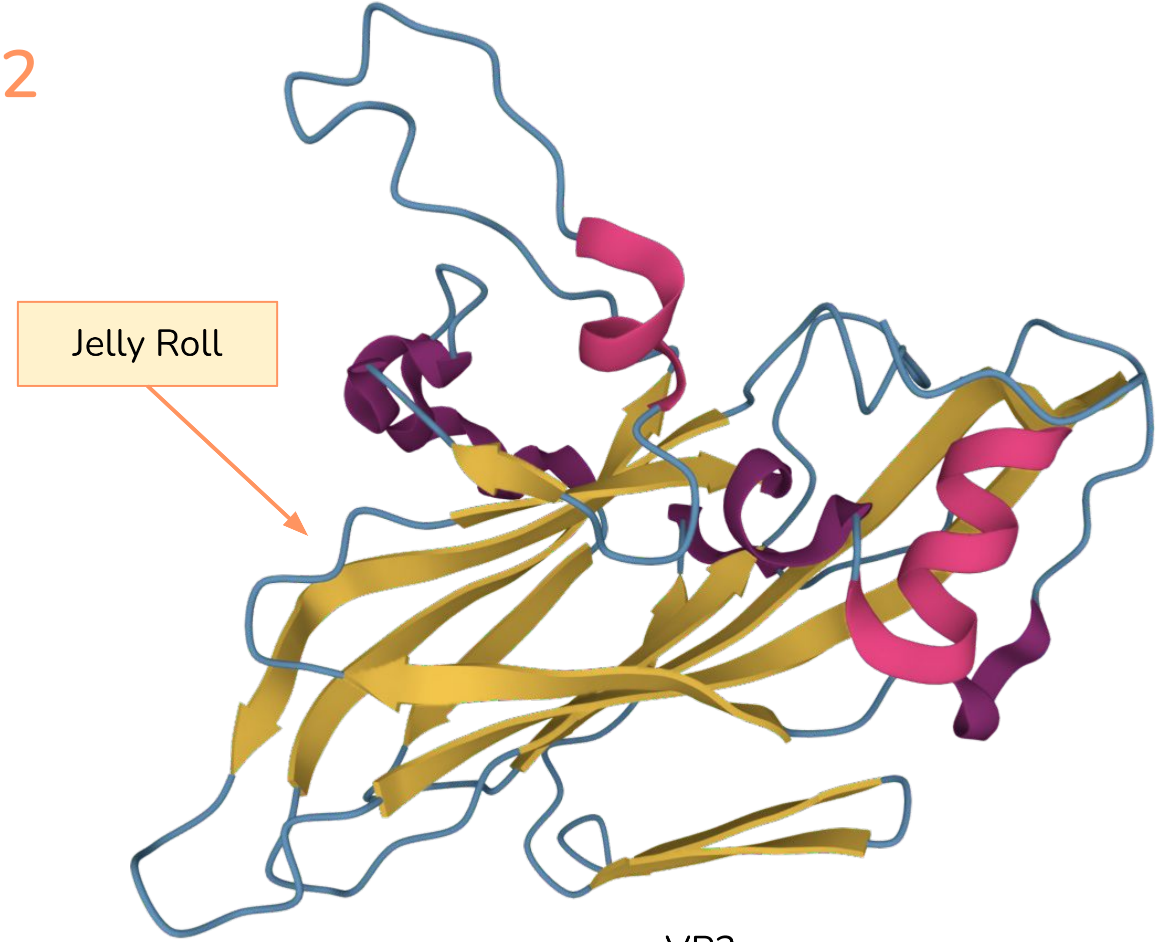


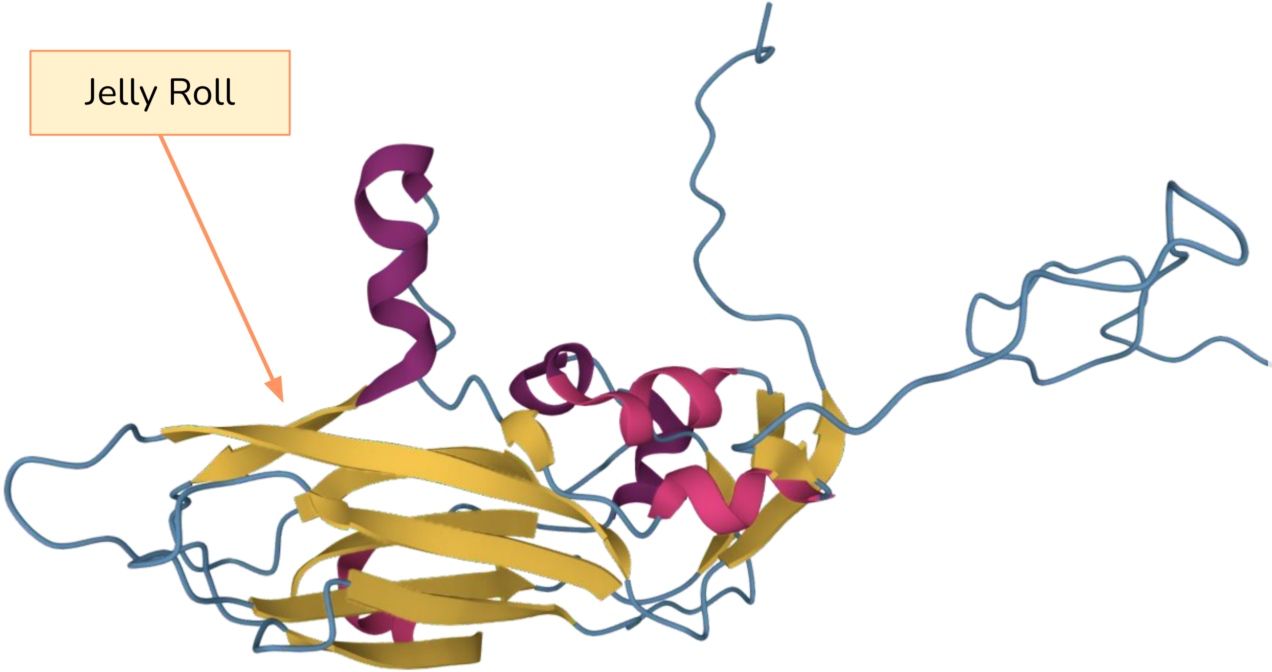
Fig. 11: Topological diagram of the secondary structures of VP2



VP2

Asymmetric unit: VP3

Jelly Roll



VP3

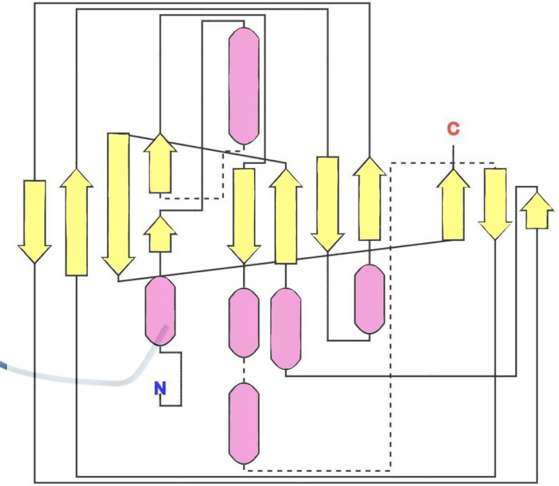


Fig. 13: Topological diagram of the secondary structures of VP3

Asymmetric unit: VP4

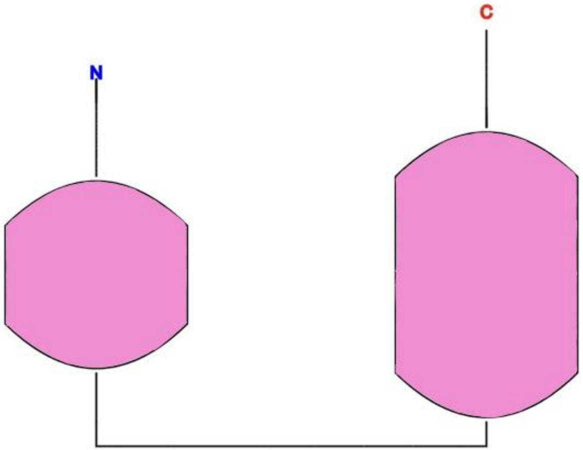
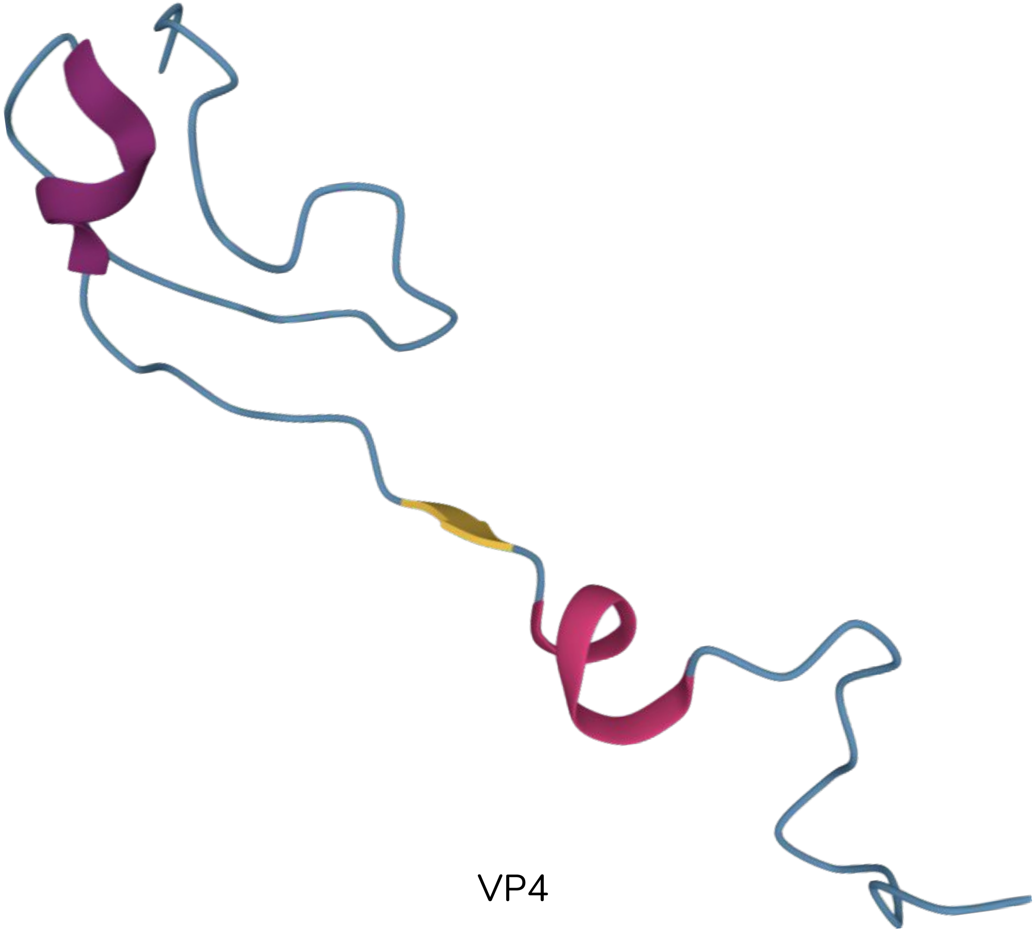


Fig. 14: Topological diagram of the secondary structures of VP4



VP4

Loops of VP1

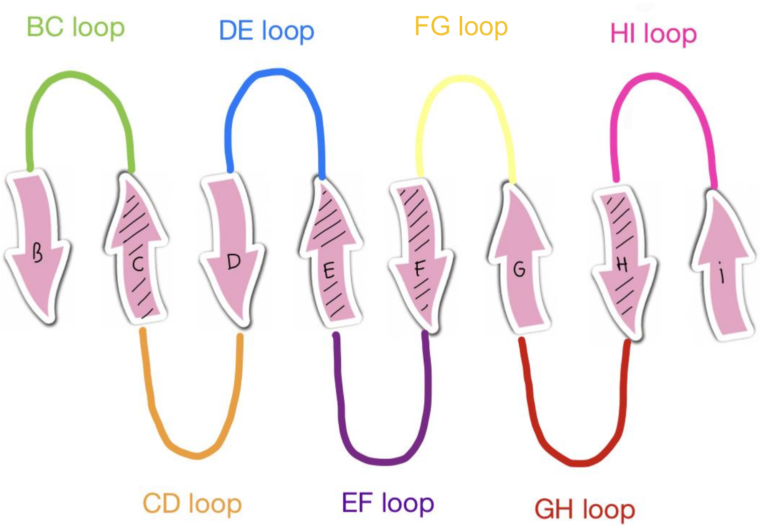
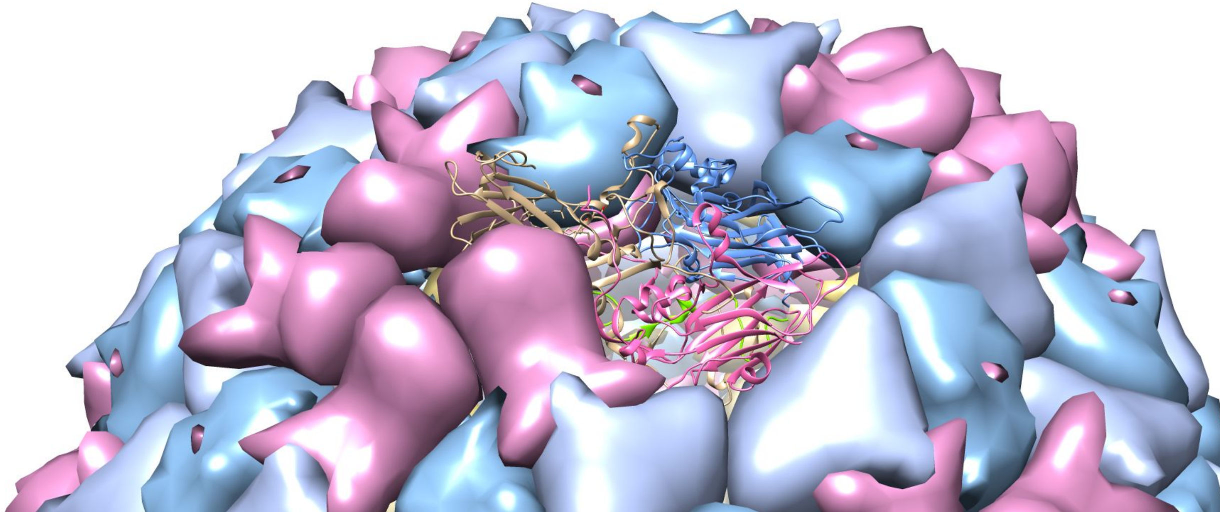


Fig. 15: Topological diagram of the loops.
Own source.

1. Relevant regions described in the bibliography



Surface features

Canyon

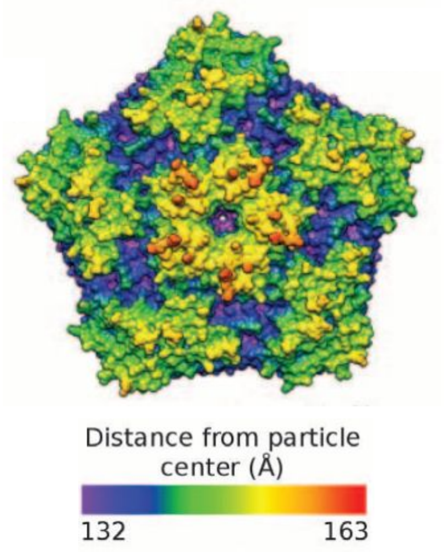
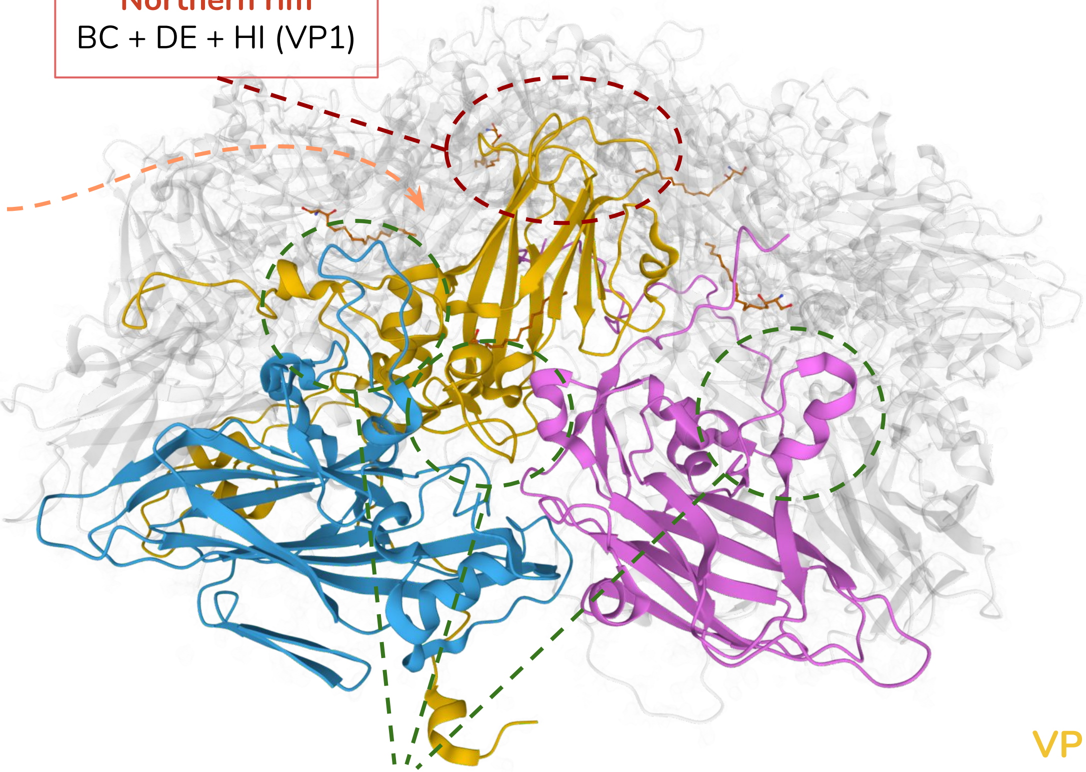


Fig. 16: Canyons.
Plevka P, et al. 2012.

Northern rim
BC + DE + HI (VP1)



Southern rim
GH (VP1) + EF/Puff (VP2) + GH/Knob (VP3)

VP1
VP2
VP3

Hydrophobic pocket

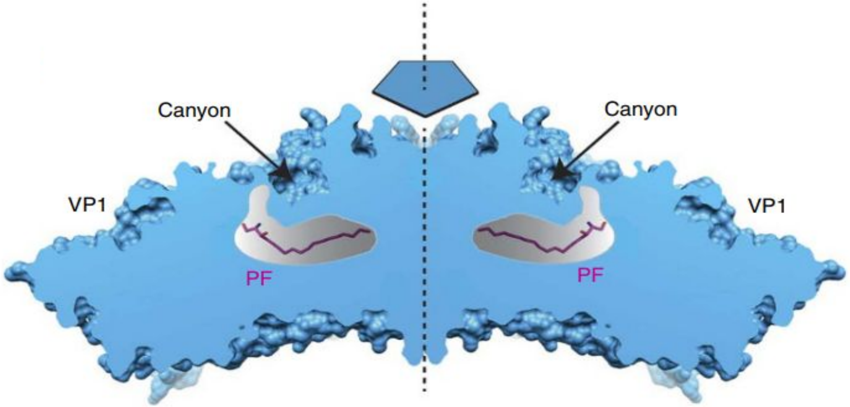
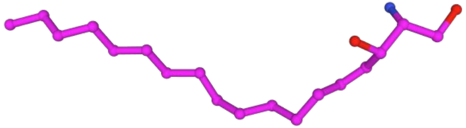


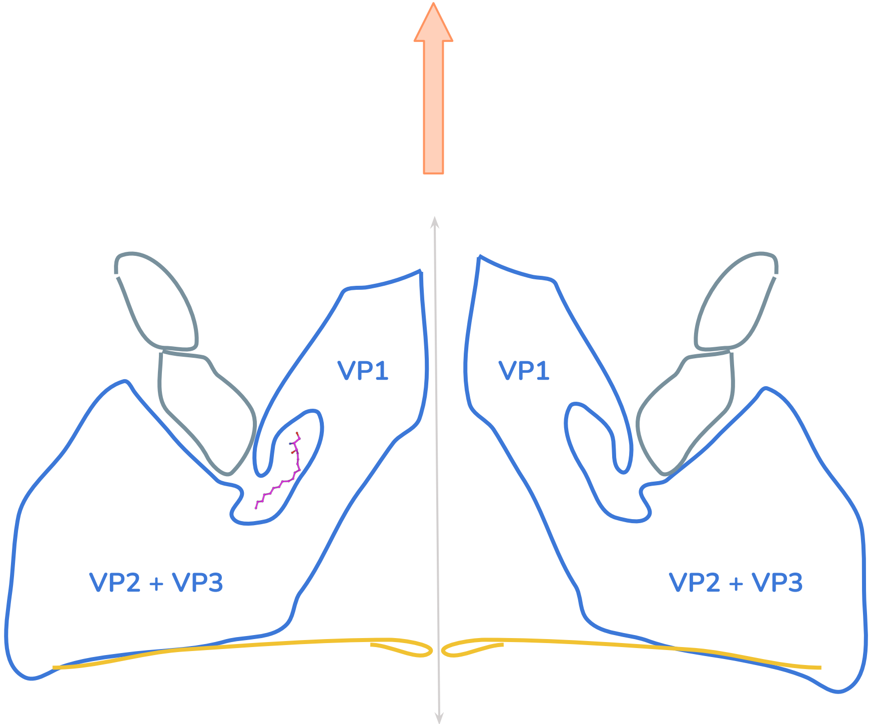
Fig. 17: Hydrophobic pocket. De Colibus L, et al. 2014.

Natural pocket factor

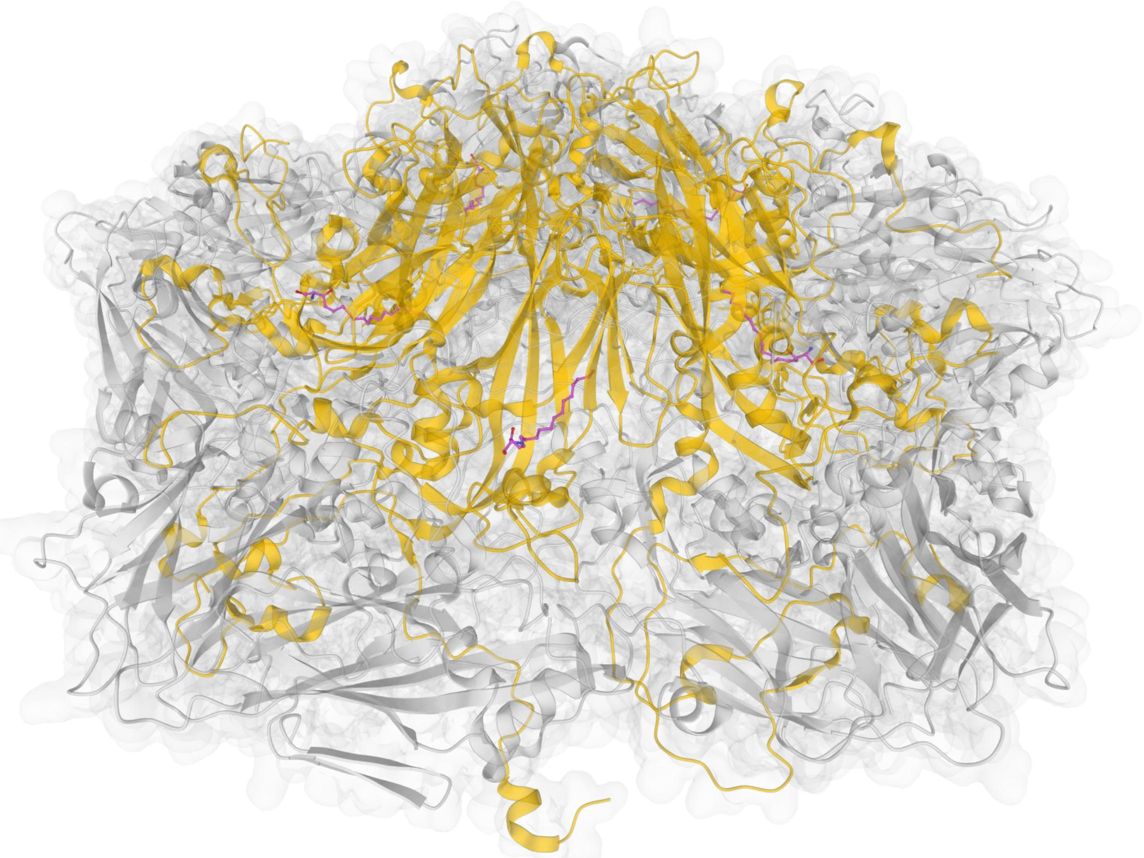


(Sphingosine)

Viral uncoating



Hydrophobic pocket



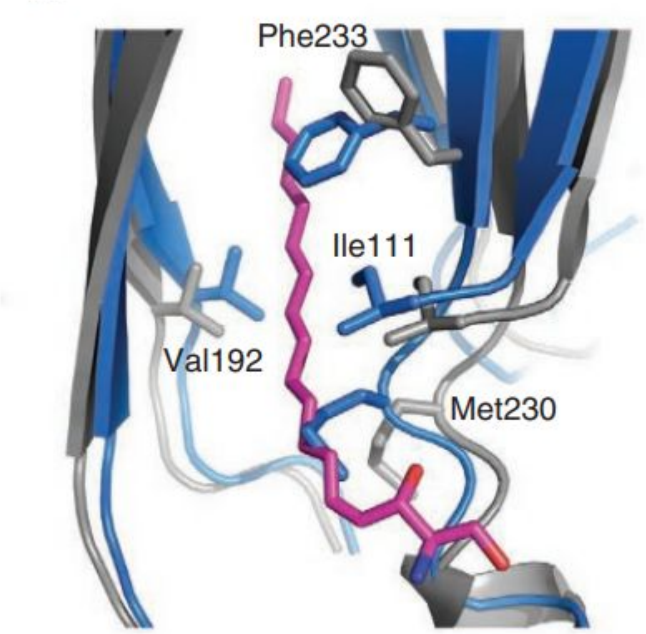


Fig. 18: Hydrophobic pocket with sphingosine. Wang X, et al. 2012.

ALD inhibitor

Imidazolidinone derivative

A: Pyridine ring

B: Phenoxy ring

C: Imidazole

+

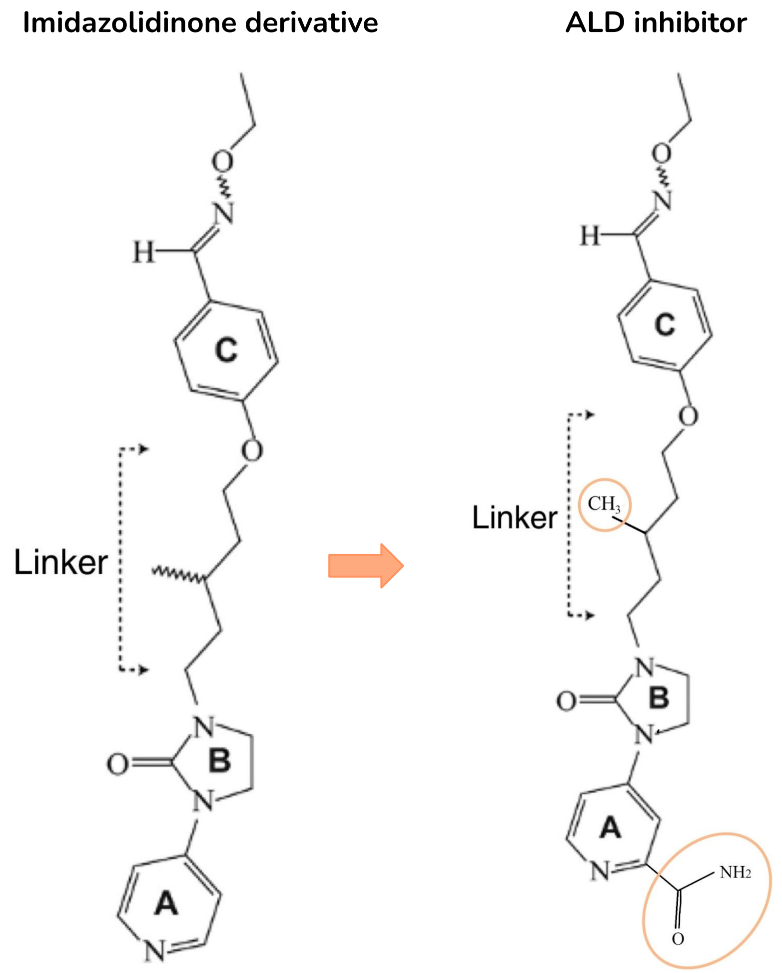
ALD inhibitor

CH_3
in the linker

$CONH_2$
in the pyridine ring

WHY?

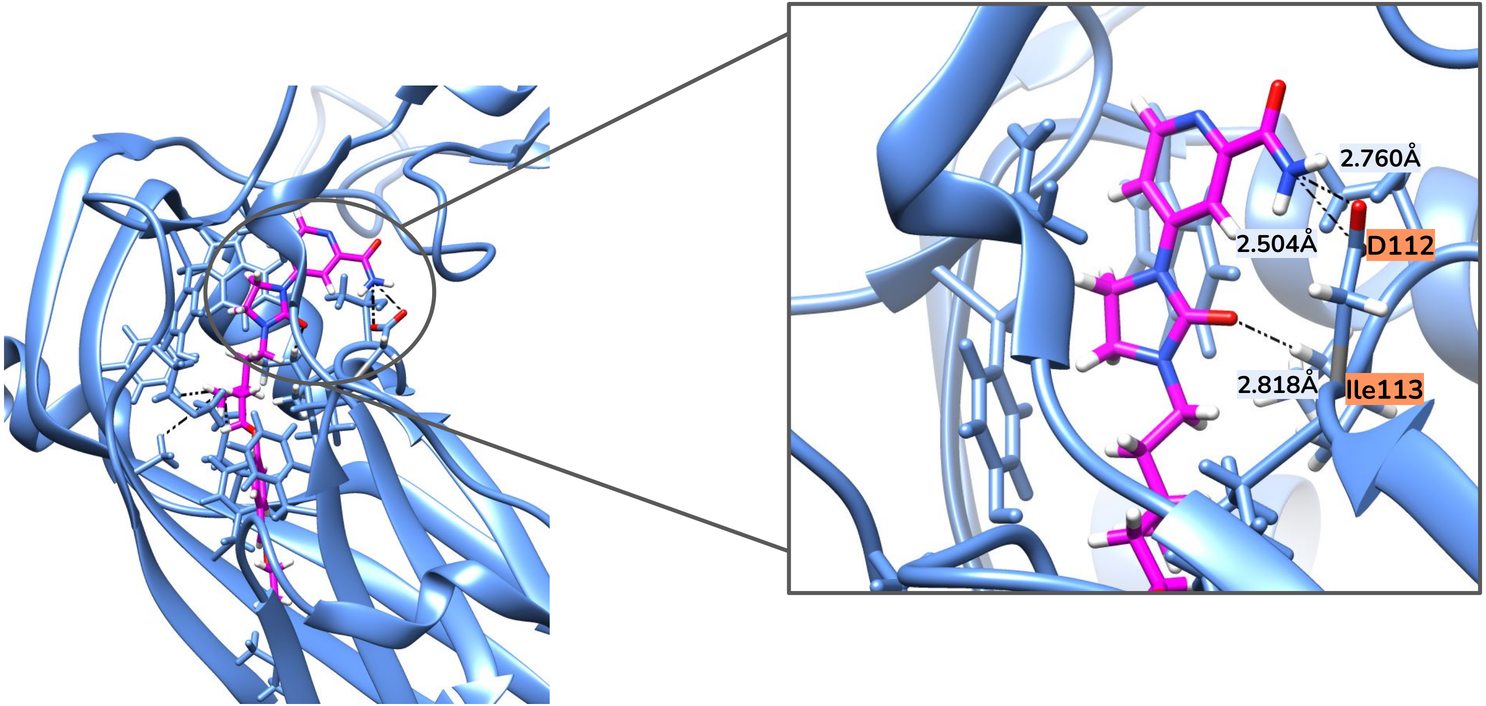
- Increases the solubility
- Enhances the affinity for the virion


Imidazolidinone derivative

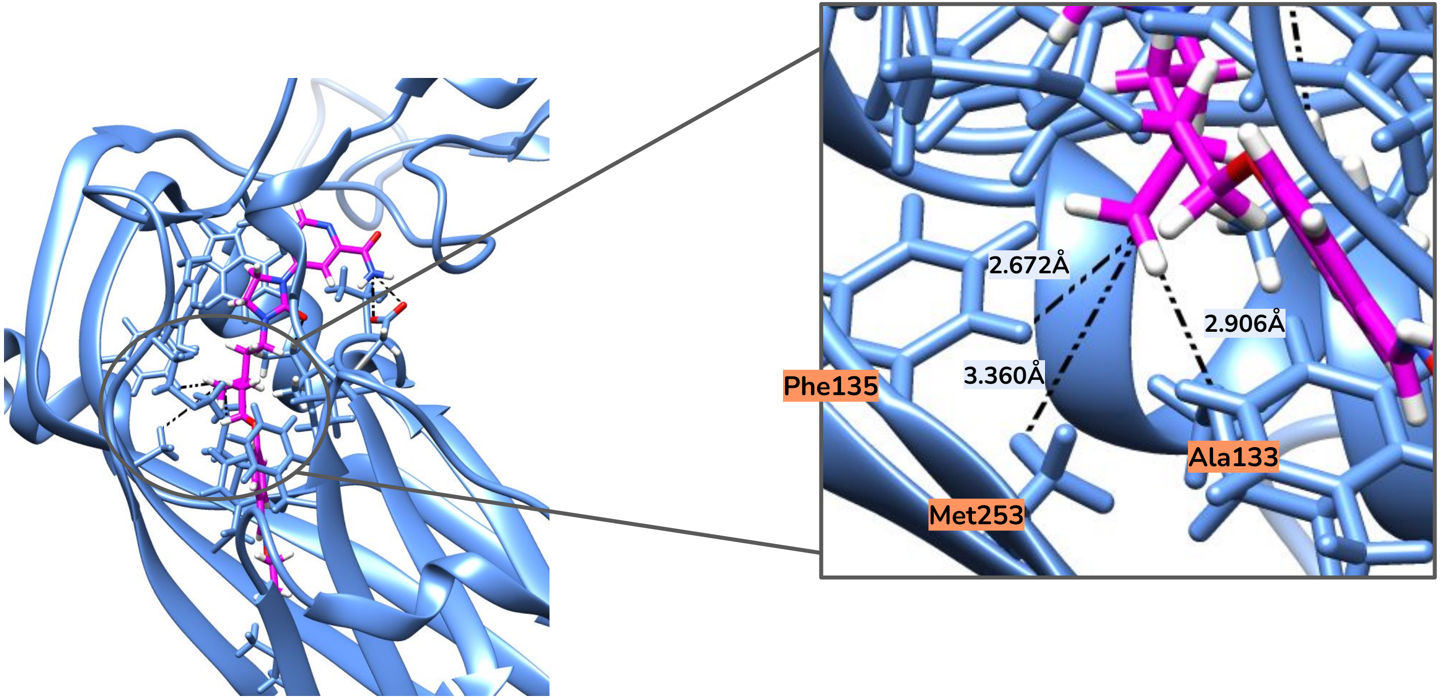
ALD inhibitor

Fig. 19: ALD inhibitor structure. Adapted from De Colibus L, et al. 2014.

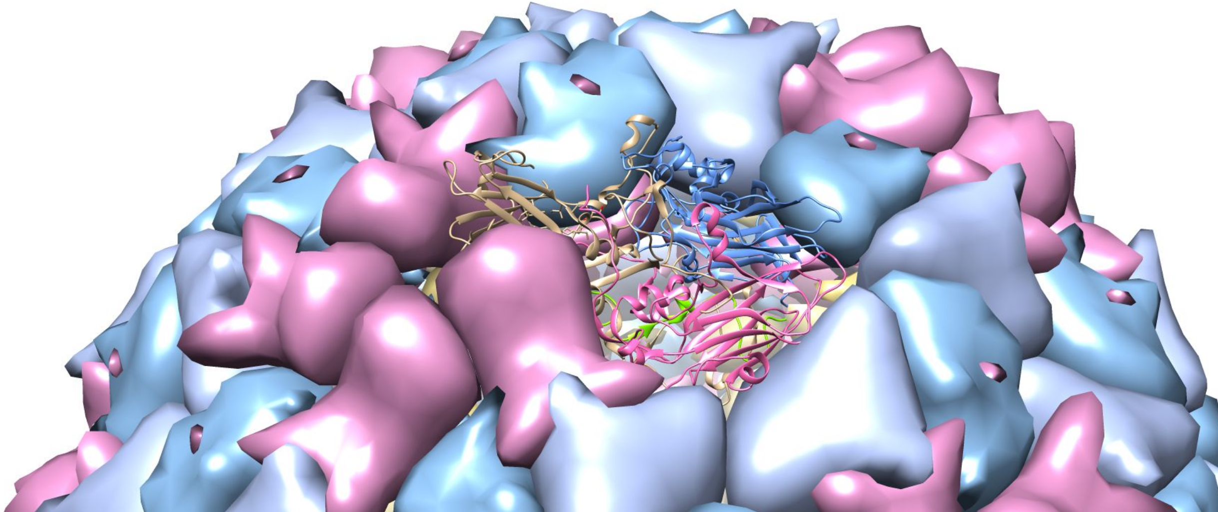
ALD inhibitor: interactions with VP1



ALD inhibitor: interactions with VP1



2. Details of specific H bonds, salt bridges… relevant for the function of the protein and their interactions with other macromolecules



Methodology

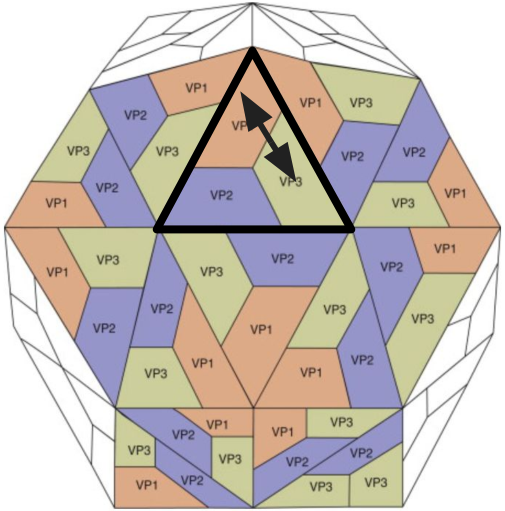
Hydrogen Bonds

1. Obtention of H bonds with **Chimera**
2. Observation of **CLUSTALW** (sequence conservation)
3. Observation of **STAMP** (structural conservation)
4. Representation of most conserved and relevant interactions

Salt Bridges

1. Obtention of **interprotomeric VP** Salt Bridges with **VMD**
2. Obtention of **intraprotomeric** Salt bridges with **VMD**
3. Observation of **CLUSTALW** and **STAMP** (conservation)
4. Representation of most conserved and relevant interactions

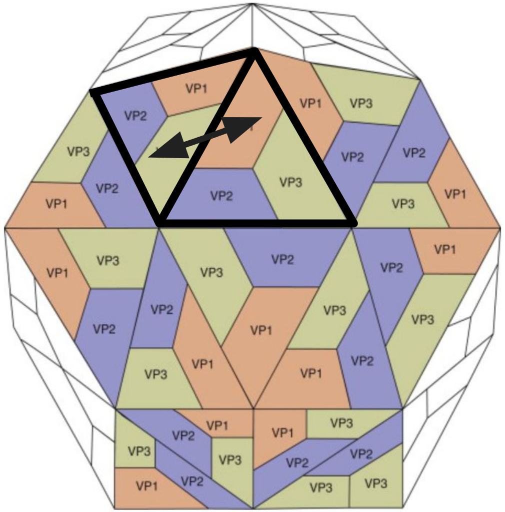
INTERACTIONS



INTRA-PROTOMERIC

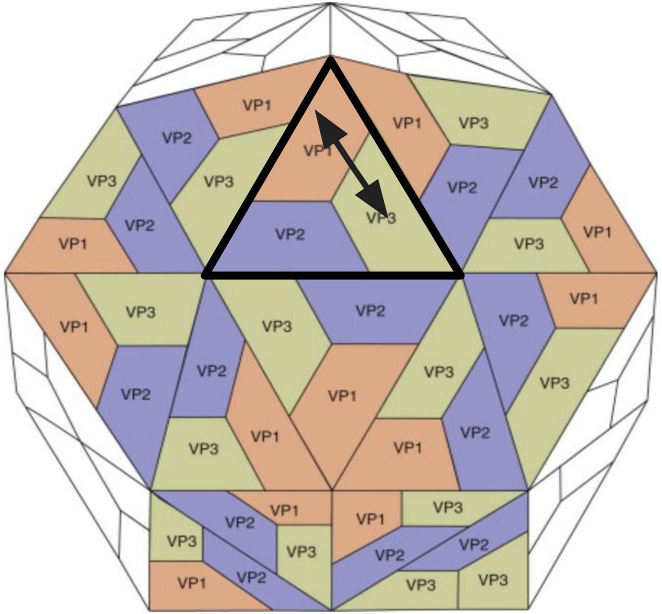
Vs

VP1-VP2
VP1-VP3
VP1-VP4
VP2-VP3

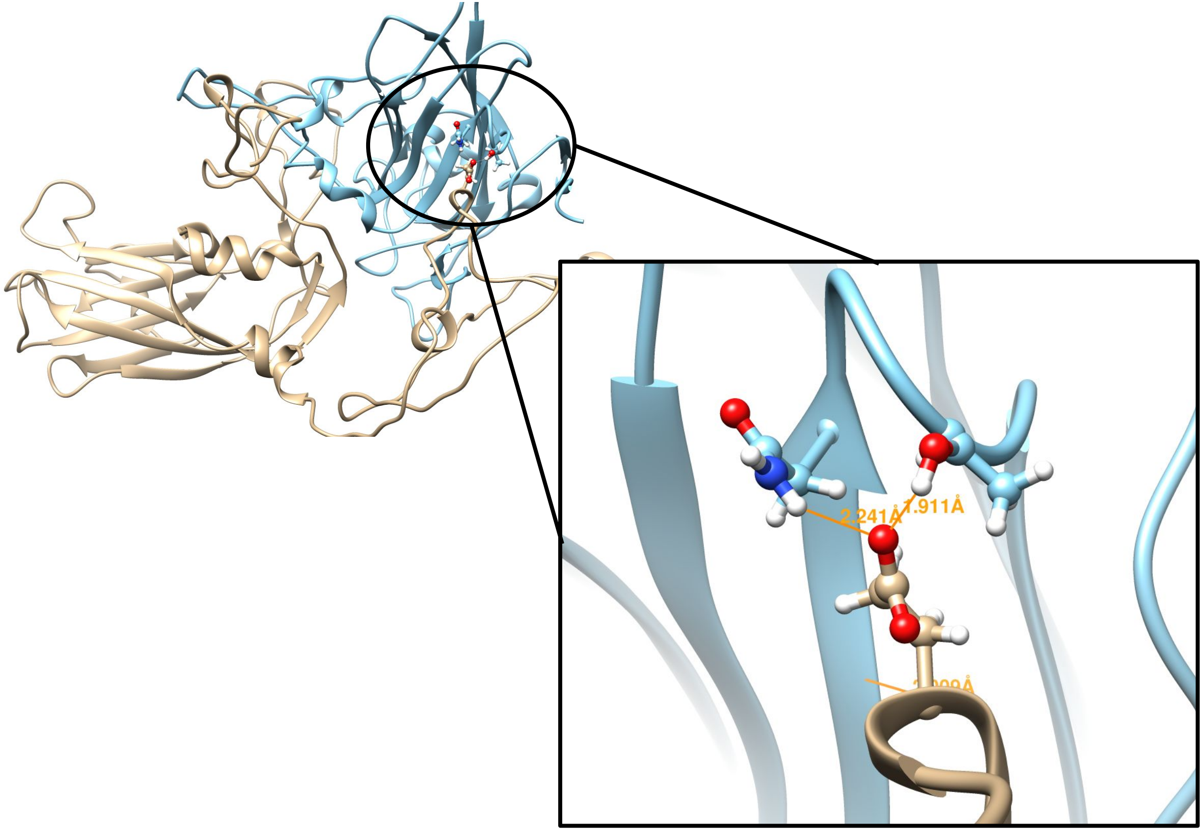


INTER-PROTOMERIC

INTRAPROTOMERIC INTERACTIONS



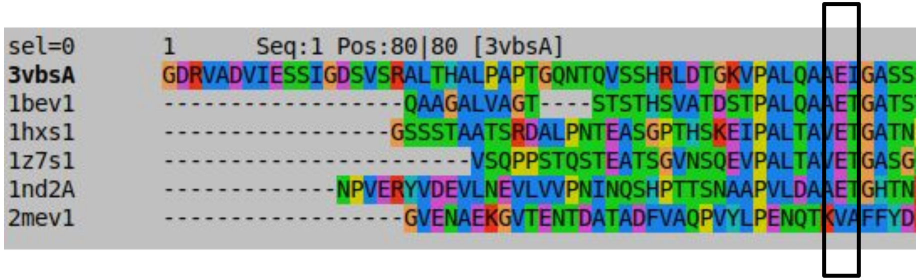
VP1 and VP2: Hydrogen Bond Conservation



VP1	VP2	Distance (Å)
Glutamic 51 O Acceptor	Asparagine 175 NH Donor	2.241
	Threonine 178 OH Donor	1.911

VP1 and VP2: Hydrogen Bond Conservation

VP1



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1hxs1        -----------------GSSSTAATSRDALPNTEASGPTHSKEIPALTAVETGATN
1z7s1        ------------------------VSQPPSTQSTEATSGVNSQEVPALTAVETGASG
1nd2A        -------------NPVERYVDEVLNEVLVVPNINQSHPTTSNAAPVLDAAETGHTN
2mev1        ------------------GVENAEKGVTENTDATADFVAQPVYLPENQTKVAFFYD
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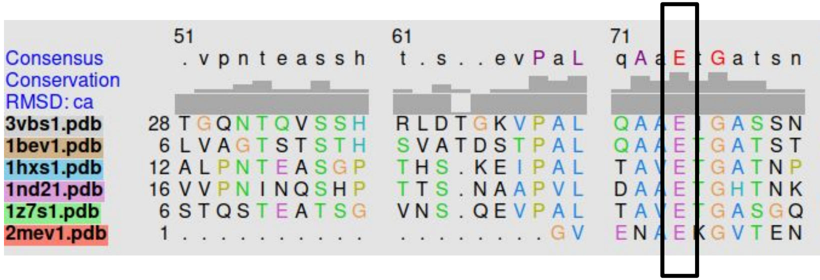

```
              51                   61                   71
Consensus      . v p n t e a s s h  t . s . . e v P a L  q A a E t G a t s n
Conservation
RMSD: ca
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1bev1.pdb    6 L V A G T S T S T H  S V A T D S T P A L  Q A A E T G A T S T
1hxs1.pdb   12 A L P N T E A S G P  T H S . K E I P A L  T A V E T G A T N P
1nd21.pdb   16 V V P N I N Q S H P  T T S . N A A P V L  D A A E T G H T N K
1z7s1.pdb    6 S T Q S T E A T S G  V N S . Q E V P A L  T A V E T G A S G Q
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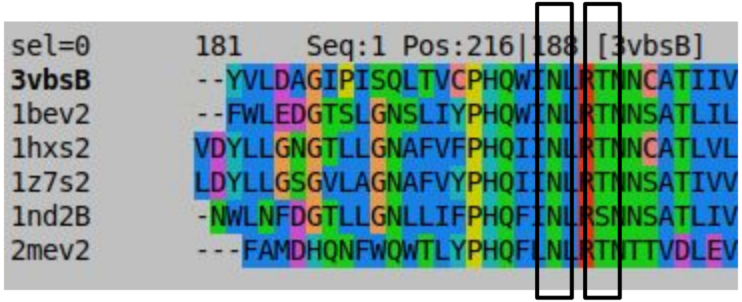
VP2



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3vbsB        --YVLDAGIPISQLTVCPHQWINLRTNNCATIIV
1bev2        --FWLEDGTSLGNSLIYPHQWINLRTNNSATLIL
1hxs2        VDYLLGNGTLLGNAFVFPHQIINLRTNNCATLVL
1z7s2        LDYLLGSGVLAGNAFVYPHQIINLRTNNSATIVV
1nd2B        -NWLNFDGTLLGNLLIFPHQFINLRSNNSATLIV
2mev2        ---FAMDHQNFWQWTLYPHQFLNLRTNTTVDLEV
```
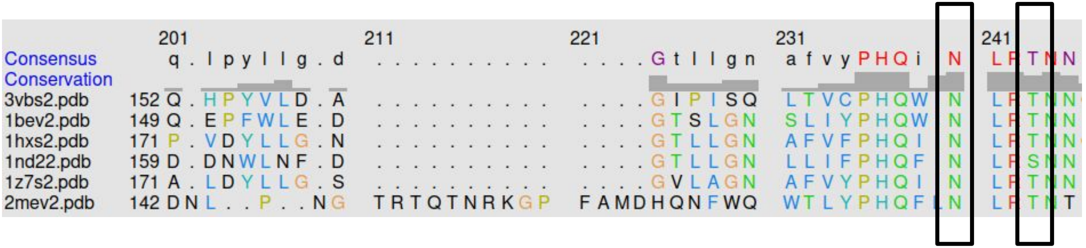
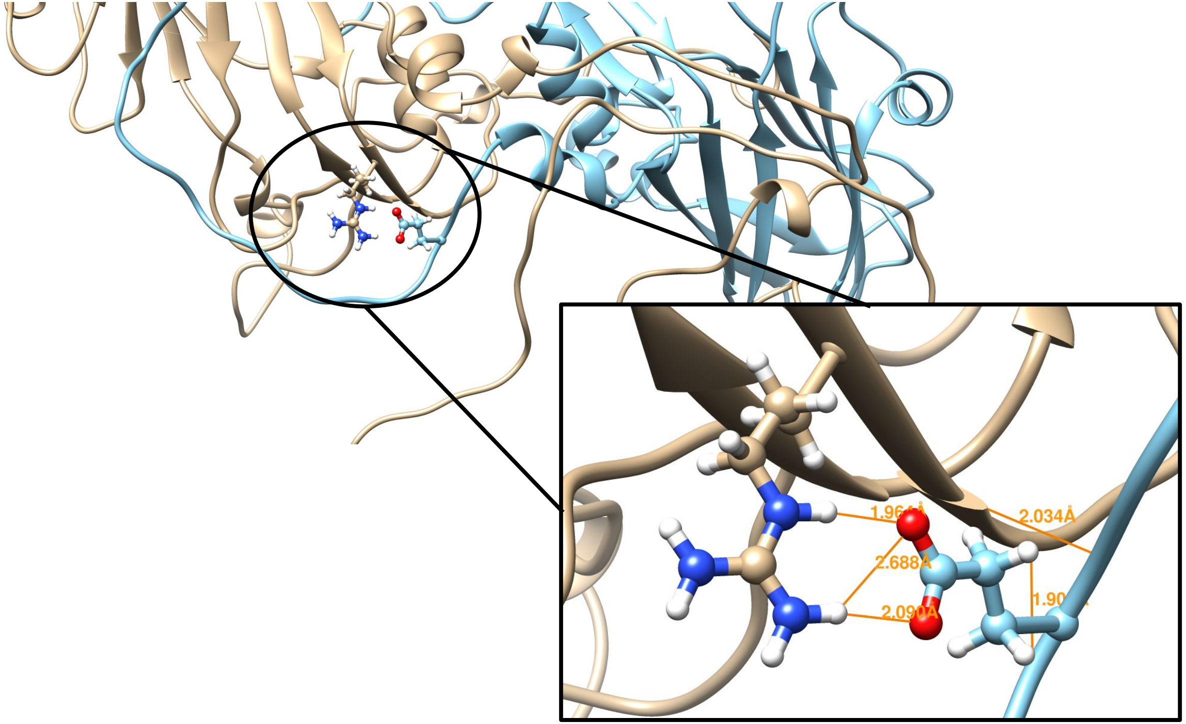



```
               201                  211                    221                   231                    241
Consensus        q . l p y l l g . d  . . . . . . . . . .  . . . . G t l l g n  a f v y P H Q i   N  L R T N N
Conservation
3vbs2.pdb    152 Q . H P Y V L D . A  . . . . . . . . . .  . . . . G I P I S Q  L T V C P H Q W   N  L R T N N
1bev2.pdb    149 Q . E P F W L E . D  . . . . . . . . . .  . . . . G T S L G N  S L I Y P H Q W   N  L R T N N
1hxs2.pdb    171 P . V D Y L L G . N  . . . . . . . . . .  . . . . G T L L G N  A F V F P H Q I   N  L R T N N
1nd22.pdb    159 D . D N W L N F . D  . . . . . . . . . .  . . . . G T L L G N  L L I F P H Q F   N  L R S N N
1z7s2.pdb    171 A . L D Y L L G . S  . . . . . . . . . .  . . . . G V L A G N  A F V Y P H Q I   N  L R T N N
2mev2.pdb    142 D N L . . P . . N G  T R T Q T N R K G P  F A M D H Q N F W Q  W T L Y P H Q F L N  L R T N T
```

VP1 and VP3: Hydrogen Bond Conservation

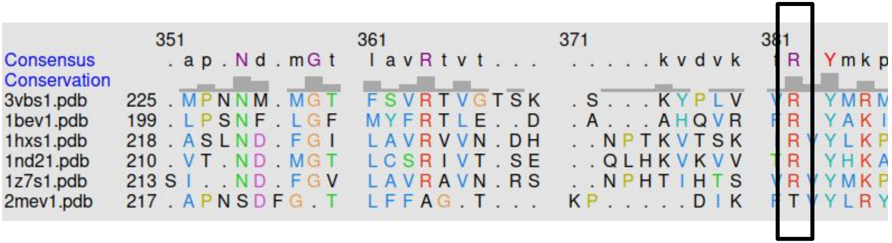


VP1	VP3	Distance (Å)
Arginine 259 NH Donor	Glutamic 39 O Acceptor	1.964
Arginine 259 NH2 Donor		2.688
		2.090

VP1 and VP3: Hydrogen Bond Conservation

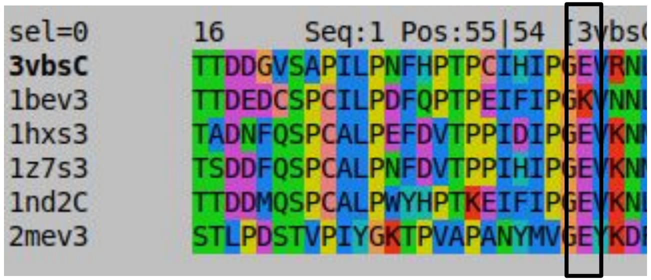
VP1

```
sel=0     241    Seq:1 Pos:299|279 [3vbsA]
3vbsA     YGAMPNNMMGTFSVRTVGT-SKSKYPLVVRIYMRMKHVRAW
1bev1     YGILPSNFLGFMYFRTL---EDAAHQVRFRIYAKIKHTSCW
1hxs1     YGAASLNDFGILAVRVVNDHNPTKVTSKIRVYLKPKHIRVW
1z7s1     YGLVSINDFGVLAVRAVNRSNPHTIHTSVRVYMKPKHIRCW
1nd2A     YGTVVTNDMGTLCSRIVTSEQLHKVKVVTRIYHKAKHTKAW
2mev1     LGIAPNSDFGTLFFAGT------KPDIKFTVYLRYKNMRVF
```

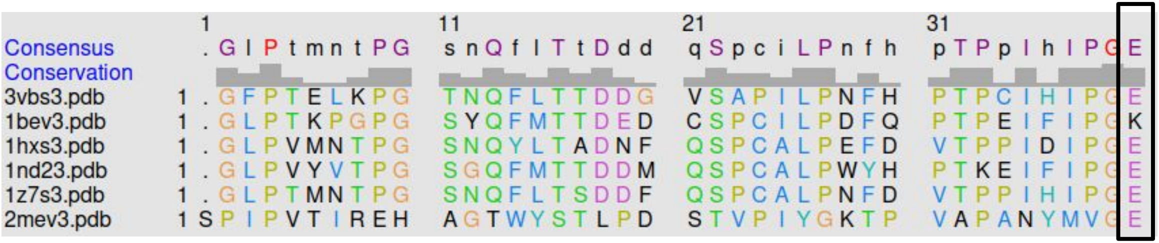


```
                       351                 361                  371                 381
Consensus              . a p . N d . m G t   l a v R t v t . . .   . . . . . k v d v k   f R   Y m k p
Conservation
3vbs1.pdb     225 . M P N N M . M G T   F S V R T V G T S K   . S . . . K Y P L V   V R   Y M R M
1bev1.pdb     199 . L P S N F . L G F   M Y F R T L E . . D   . A . . . A H Q V R   F R   Y A K I
1hxs1.pdb     218 . A S L N D . F G I   L A V R V V N . D H   . . N P T K V T S K   I R V Y L K P
1nd21.pdb     210 . V T . N D . M G T   L C S R I V T . S E   . . Q L H K V K V V   T R   Y H K A
1z7s1.pdb     213 S I . . N D . F G V   L A V R A V N . R S   . . N P H T I H T S   V R V Y M K P
2mev1.pdb     217 . A P N S D F G . T   L F F A G . T . . .   K P . . . . . D I K   F T V Y L R Y
```

VP3

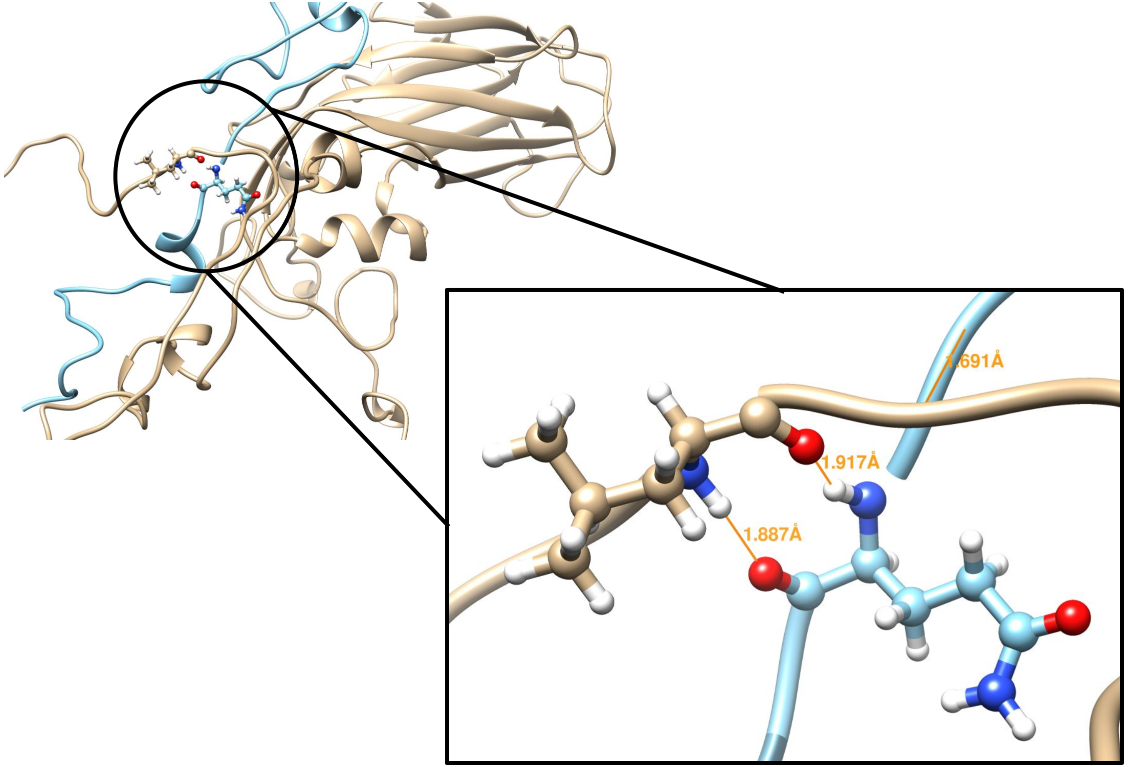


```
sel=0     16        Seq:1 Pos:55|54 [3vbs
3vbsC     TTDDGVSAPILPNFHPTPCIHIPGEVRN
1bev3     TTDEDCSPCILPDFQPTPEIFIPGKVNN
1hxs3     TADNFQSPCALPEFDVTPPIDIPGEVKN
1z7s3     TSDDFQSPCALPNFDVTPPIHIPGEVKN
1nd2C     TTDDMQSPCALPWYHPTKEIFIPGEVKN
2mev3     STLPDSTVPIYGKTPVAPANYMVGEYKD
```



```
                       1                     11                    21                    31
Consensus              . G I P t m n t P G   s n Q f l T t D d d   q S p c i L P n f h   p T P p l h l P G E
Conservation
3vbs3.pdb       1 . G F P T E L K P G   T N Q F L T T D D G   V S A P I L P N F H   P T P C I H I P G E
1bev3.pdb       1 . G L P T K P G P G   S Y Q F M T T D E D   C S P C I L P D F Q   P T P E I F I P G K
1hxs3.pdb       1 . G L P V M N T P G   S N Q Y L T A D N F   Q S P C A L P E F D   V T P P I D I P G E
1nd23.pdb       1 . G L P V Y V T P G   S G Q F M T T D D M   Q S P C A L P W Y H   P T K E I F I P G E
1z7s3.pdb       1 . G L P T M N T P G   S N Q F L T S D D F   Q S P C A L P N F D   V T P P I H I P G E
2mev3.pdb       1 S P I P V T I R E H   A G T W Y S T L P D   S T V P I Y G K T P   V A P A N Y M V G E
```

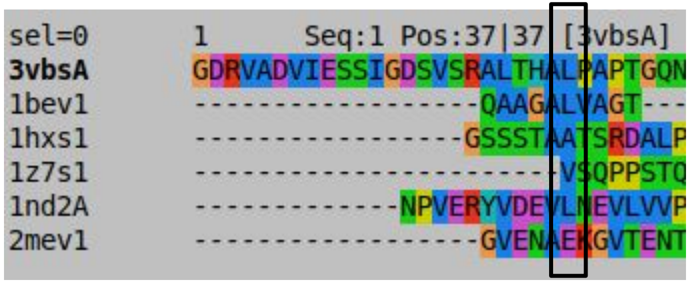
VP1 and VP4: Hydrogen Bond Conservation



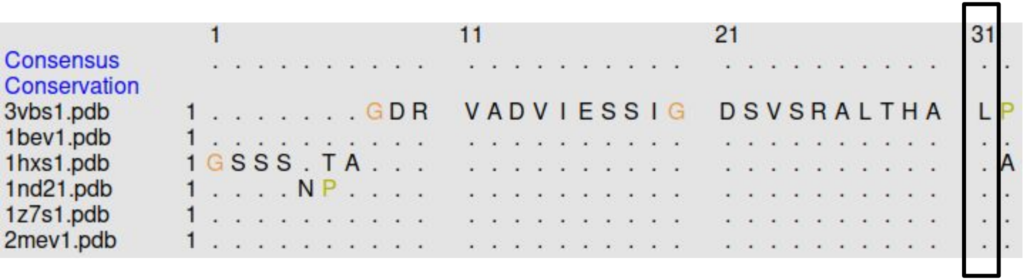
VP1	VP4	Distance (Å)
Leucine 24 O Acceptor	Glutamine 37 NH Donor	1.917
Leucine 24 NH Donor	Glutamine 37 O Acceptor	1.887

VP1 and VP4: Hydrogen Bond Conservation

VP1

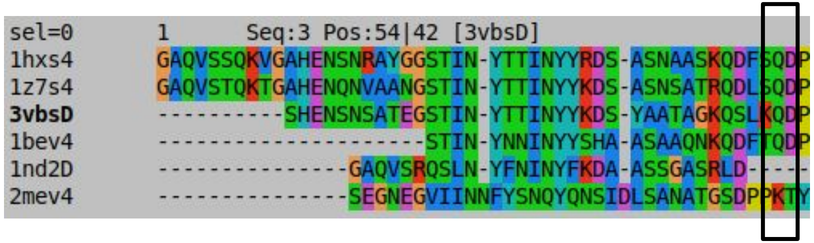


```
sel=0         1      Seq:1 Pos:37|37 [3vbsA]
3vbsA         GDRVADVIESSIGDSVSRALTHALPAPTGQN
1bev1         ------------------QAAGALVAGT---
1hxs1         -----------------GSSSTAATSRDALP
1z7s1         ----------------------VSQPPSTQ
1nd2A         ------------NPVERYVDEVLNEVLVVP
2mev1         ------------------GVENAEKGVTENT
```

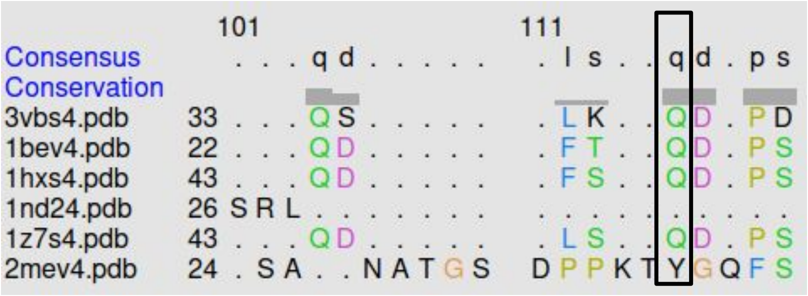


```
                   1                    11                   21                   31
Consensus          . . . . . . . . . .  . . . . . . . . . .  . . . . . . . . . .  . .
Conservation
3vbs1.pdb     1    . . . . . . . G D R  V A D V I E S S I G  D S V S R A L T H A  L P
1bev1.pdb     1    . . . . . . . . . .  . . . . . . . . . .  . . . . . . . . . .  . .
1hxs1.pdb     1    G S S S . T A . . .  . . . . . . . . . .  . . . . . . . . . .  . A
1nd21.pdb     1    . . . . N P . . . .  . . . . . . . . . .  . . . . . . . . . .  . .
1z7s1.pdb     1    . . . . . . . . . .  . . . . . . . . . .  . . . . . . . . . .  . .
2mev1.pdb     1    . . . . . . . . . .  . . . . . . . . . .  . . . . . . . . . .  . .
```

VP4

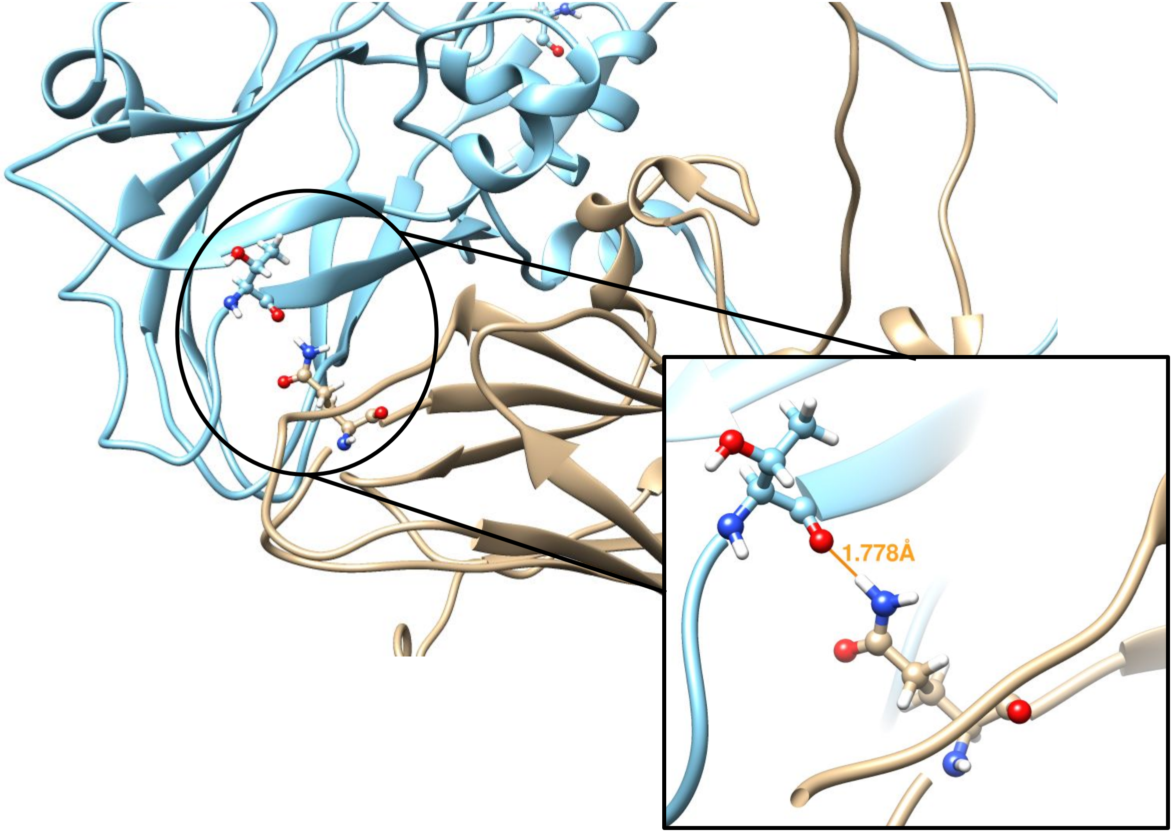


```
sel=0        1       Seq:3 Pos:54|42 [3vbsD]
1hxs4        GAQVSSQKVGAHENSNRAYGGSTIN-YTTINYYRDS-ASNAASKQDFSQDP
1z7s4        GAQVSTQKTGAHENQNVAANGSTIN-YTTINYYKDS-ASNSATRQDLSQDP
3vbsD        ----------SHENSNSATEGSTIN-YTTINYYKDS-YAATAGKQSLKQDP
1bev4        ---------------------STIN-YNNINYYSHA-ASAAQNKQDFTQDP
1nd2D        --------------GAQVSRQSLN-YFNINYFKDA-ASSGASRLD-----
2mev4        --------------SEGNEGVIINNFYSNQYQNSIDLSANATGSDPPKTY
```



```
                          101                  111
Consensus                 . . . q d . . . . .  . l s . . q d . p s
Conservation
3vbs4.pdb     33          . . . Q S . . . . .  . L K . . Q D . P D
1bev4.pdb     22          . . . Q D . . . . .  . F T . . Q D . P S
1hxs4.pdb     43          . . . Q D . . . . .  . F S . . Q D . P S
1nd24.pdb     26          S R L . . . . . . .  . . . . . . . . . .
1z7s4.pdb     43          . . . Q D . . . . .  . L S . . Q D . P S
2mev4.pdb     24          . S A . . N A T G S  D P P K T Y G Q F S
```

VP2 and VP3: Hydrogen Bond Conservation

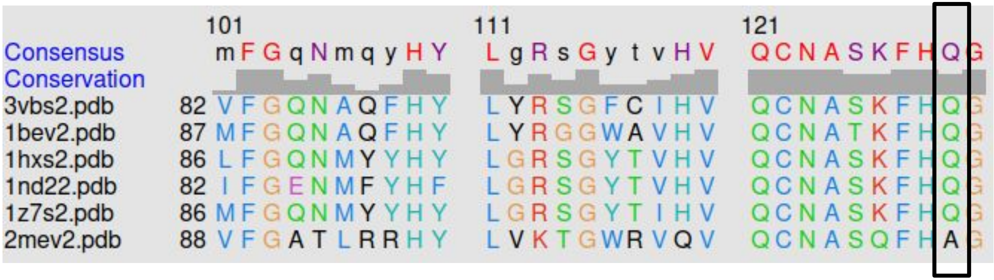


VP2	VP3	Distance (Å)
Glutamine 110 NH Donor	Threonine 211 O Acceptor	1.778

VP2 and VP3: Hydrogen Bond Conservation

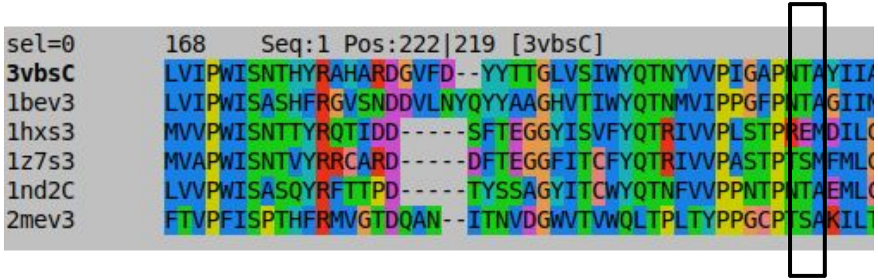
VP2

```
sel=0        96      Seq:1 Pos:140|130 [3vbsB]
3vbsB        NAQFHYLYRSGFCIHVQCNASKFHQGALLVAVL
1bev2        NAQFHYLYRGGWAVHVQCNATKFHQGTLLVLAI
1hxs2        NMYYHYLGRSGYTVHVQCNASKFHQGALGVFAV
1z7s2        NMYYHYLGRSGYTIHVQCNASKFHQGALGVFLI
1nd2B        NMFYHFLGRSGYTVHVQCNASKFHQGTLLVVMI
2mev2        TLRRHYLVKTGWRVQVQCNASQFHAGSLLVFMA
```

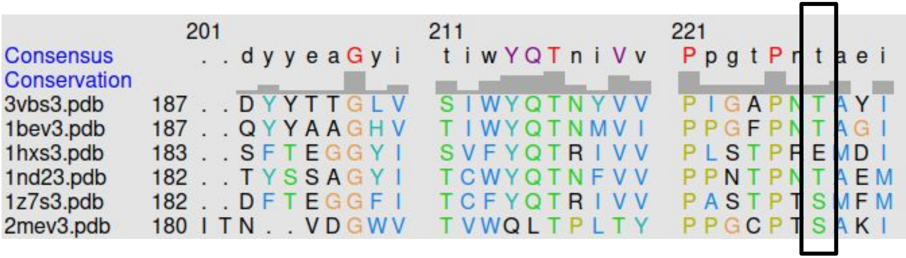


```
                 101                  111                  121
Consensus        m F G q N m q y H Y  L g R s G y t v H V  Q C N A S K F H Q G
Conservation
3vbs2.pdb    82  V F G Q N A Q F H Y  L Y R S G F C I H V  Q C N A S K F H Q G
1bev2.pdb    87  M F G Q N A Q F H Y  L Y R G G W A V H V  Q C N A T K F H Q G
1hxs2.pdb    86  L F G Q N M Y Y H Y  L G R S G Y T V H V  Q C N A S K F H Q G
1nd22.pdb    82  I F G E N M F Y H F  L G R S G Y T V H V  Q C N A S K F H Q G
1z7s2.pdb    86  M F G Q N M Y Y H Y  L G R S G Y T I H V  Q C N A S K F H Q G
2mev2.pdb    88  V F G A T L R R H Y  L V K T G W R V Q V  Q C N A S Q F H A G
```

VP3

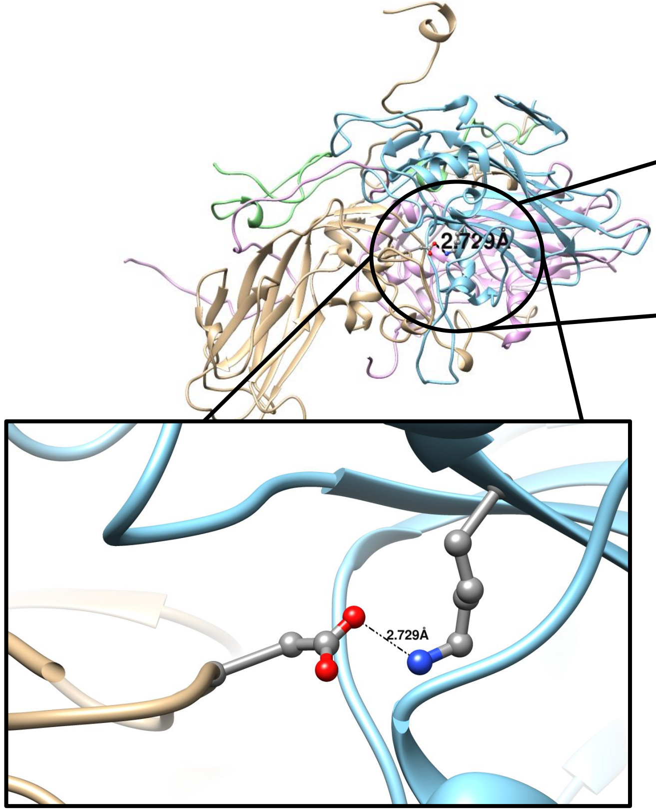


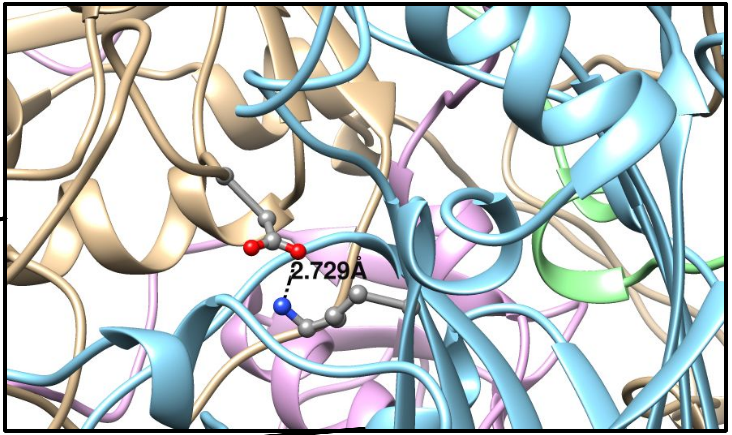
```
sel=0      168    Seq:1 Pos:222|219 [3vbsC]
3vbsC      LVIPWISNTHYRAHARDGVFD--YYTTGLVSIWYQTNYVVPIGAPNTAYII
1bev3      LVIPWISASHFRGVSNDDVLNYQYYAAGHVTIWYQTNMVIPPGFPNTAGII
1hxs3      MVVPWISNTTYRQTIDD-----SFTEGGYISVFYQTRIVVPLSTPREMDIL
1z7s3      MVAPWISNTVYRRCARD-----DFTEGGFITCFYQTRIVVPASTPTSMFML
1nd2C      LVVPWISASQYRFTTPD-----TYSSAGYITCWYQTNFVVPPNTPNTAEML
2mev3      FTVPFISPTHFRMVGTDQAN--ITNVDGWVTVWQLTPLTYPPGCPTSAKIL
```

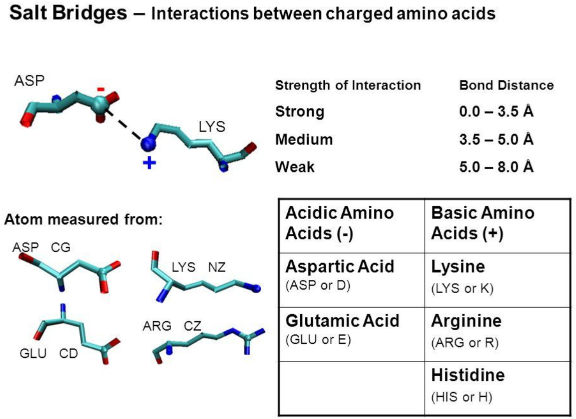


```
                   201                   211                   221
Consensus          . . d y y e a G y i   t i w Y Q T n i V v   P p g t P n t a e i
Conservation
3vbs3.pdb    187   . . D Y Y T T G L V   S I W Y Q T N Y V V   P I G A P N T A Y I
1bev3.pdb    187   . . Q Y Y A A G H V   T I W Y Q T N M V I   P P G F P N T A G I
1hxs3.pdb    183   . . S F T E G G Y I   S V F Y Q T R I V V   P L S T P R E M D I
1nd23.pdb    182   . . T Y S S A G Y I   T C W Y Q T N F V V   P P N T P N T A E M
1z7s3.pdb    182   . . D F T E G G F I   T C F Y Q T R I V V   P A S T P T S M F M
2mev3.pdb    180   I T N . . V D G W V   T V W Q L T P L T Y   P P G C P T S A K I
```

VP1 and VP2: Salt Bridges







Salt Bridges – Interactions between charged amino acids

Strength of Interaction	Bond Distance
Strong	0.0 – 3.5 Å
Medium	3.5 – 5.0 Å
Weak	5.0 – 8.0 Å

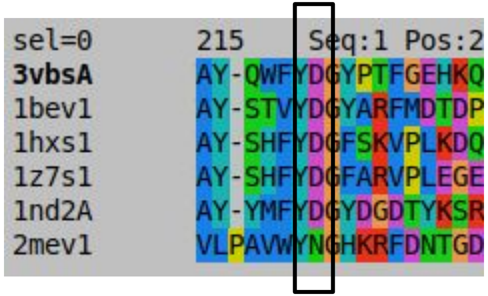
Atom measured from:

Acidic Amino Acids (-)	Basic Amino Acids (+)
Aspartic Acid (ASP or D)	**Lysine** (LYS or K)
Glutamic Acid (GLU or E)	**Arginine** (ARG or R)
	Histidine (HIS or H)

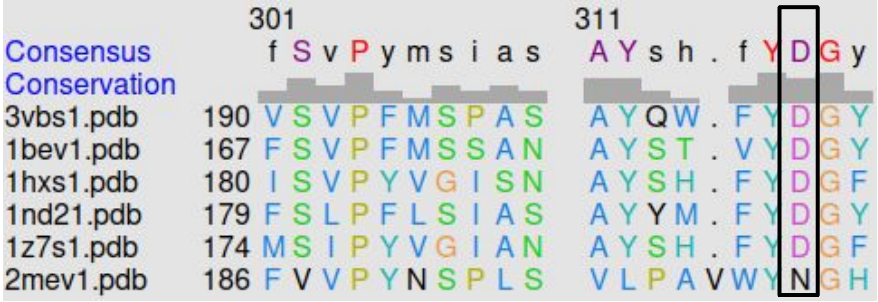
Aspartic (-) 206 of VP1 +
Lysine (+) 72 of VP2
2.729 Å

VP1 and VP2: Salt Bridges

VP1



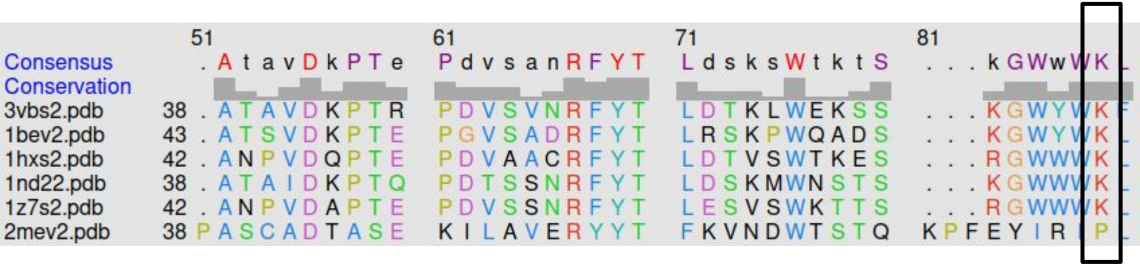
```
sel=0          215     Seq:1 Pos:2
3vbsA          AY-QWFYDGYPTFGEHKQ
1bev1          AY-STVYDGYARFMDTDP
1hxs1          AY-SHFYDGFSKVPLKDQ
1z7s1          AY-SHFYDGFARVPLEGE
1nd2A          AY-YMFYDGYDGDTYKSR
2mev1          VLPAVWYNGHKRFDNTGD
```



```
                      301                    311
Consensus             f S v P y m s i a s    A Y s h . f Y D G y
Conservation
3vbs1.pdb        190  V S V P F M S P A S    A Y Q W . F Y D G Y
1bev1.pdb        167  F S V P F M S S A N    A Y S T . V Y D G Y
1hxs1.pdb        180  I S V P Y V G I S N    A Y S H . F Y D G F
1nd21.pdb        179  F S L P F L S I A S    A Y Y M . F Y D G Y
1z7s1.pdb        174  M S I P Y V G I A N    A Y S H . F Y D G F
2mev1.pdb        186  F V V P Y N S P L S    V L P A V W Y N G H
```

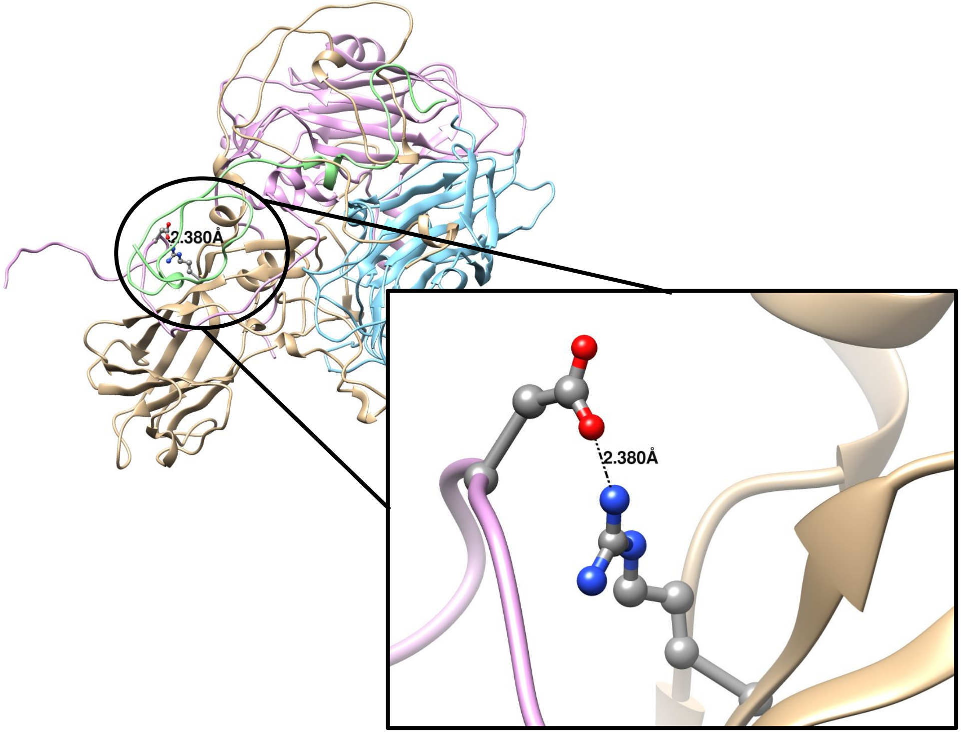
VP2

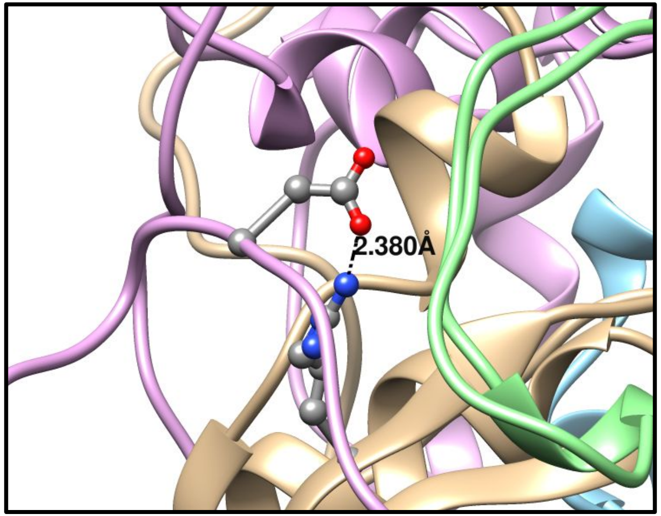
```
sel=0          47     Seq:1 Pos:86|78 [3vbsB]
3vbsB          DKPTRPDVSVNRFYTLDTKLWEKSSKGWYWK--
1bev2          DKPTEPGVSADRFYTLRSKPWQADSKGWYWK--
1hxs2          DQPTEPDVAACRFYTLDTVSWTKESRGWWWK--
1z7s2          DAPTEPDVSSNRFYTLESVSWKTTSRGWWWK--
1nd2B          DKPTQPDTSSNRFYTLDSKMWNSTSKGWWWK--
2mev2          DTASEKILAVERYYTFKVNDWTSTQKPFEYIRI
```



```
                    51                    61                    71                    81
Consensus           . A t a v D k P T e   P d v s a n R F Y T   L d s k s W t k t S   . . . k G W w W K L
Conservation
3vbs2.pdb       38  . A T A V D K P T R   P D V S V N R F Y T   L D T K L W E K S S   . . . K G W Y W K F
1bev2.pdb       43  . A T S V D K P T E   P G V S A D R F Y T   L R S K P W Q A D S   . . . K G W Y W K L
1hxs2.pdb       42  . A N P V D Q P T E   P D V A A C R F Y T   L D T V S W T K E S   . . . R G W W W K L
1nd22.pdb       38  . A T A I D K P T Q   P D T S S N R F Y T   L D S K M W N S T S   . . . K G W W W K L
1z7s2.pdb       42  . A N P V D A P T E   P D V S S N R F Y T   L E S V S W K T T S   . . . R G W W W K L
2mev2.pdb       38  P A S C A D T A S E   K I L A V E R Y Y T   F K V N D W T S T Q   K P F E Y I R I P L
```

VP1 and VP3: Salt Bridges

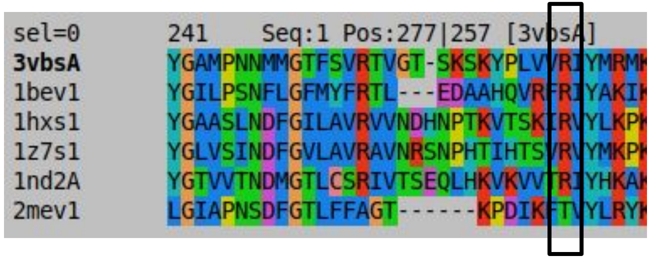




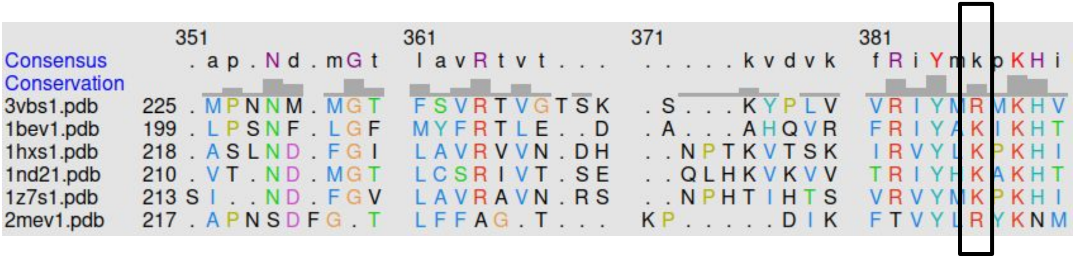
Arginine (+) 254 of VP1 +
Aspartic (-) 18 of VP3
2.380 Å

VP1 and VP3: Salt Bridges

VP1



```
sel=0        241    Seq:1 Pos:277|257 [3vbsA]
3vbsA        YGAMPNNMMGTFSVRTVGT-SKSKYPLVVRIYMRM
1bev1        YGILPSNFLGFMYFRTL---EDAAHQVRFRIYAKI
1hxs1        YGAASLNDFGILAVRVVNDHNPTKVTSKIRVYLKP
1z7s1        YGLVSINDFGVLAVRAVNRSNPHTIHTSVRVYMKP
1nd2A        YGTVVTNDMGTLCSRIVTSEQLHKVKVVTRIYHKA
2mev1        LGIAPNSDFGTLFFAGT------KPDIKFTVYLRY
```



```
                    351              361              371              381
Consensus          . a p . N d . m G t   l a v R t v t . . .   . . . . . . k v d v k   f R i Y m k p K H i
Conservation
3vbs1.pdb     225 . M P N N M . M G T   F S V R T V G T S K   . S . . . K Y P L V     V R I Y M R M K H V
1bev1.pdb     199 . L P S N F . L G F   M Y F R T L E . . D   . A . . . A H Q V R     F R I Y A K I K H T
1hxs1.pdb     218 . A S L N D . F G I   L A V R V V N . D H   . . N P T K V T S K     I R V Y L K P K H I
1nd21.pdb     210 . V T . N D . M G T   L C S R I V T . S E   . . Q L H K V K V V     T R I Y H K A K H T
1z7s1.pdb     213 S I . . N D . F G V   L A V R A V N . R S   . . N P H T I H T S     V R V Y M K P K H I
2mev1.pdb     217 . A P N S D F G . T   L F F A G . T . . .   K P . . . . . . D I K   F T V Y L R Y K N M
```

VP3

```
sel=0         1        Seq:1 Pos:30|29
3vbsC         -GFPTELKPGTNQFLTTDDGVSA
1bev3         -GLPTKPGPGSYQFMTTDEDCSP
1hxs3         -GLPVMNTPGSNQYLTADNFQSP
1z7s3         -GLPTMNTPGSNQFLTSDDFQSP
1nd2C         -GLPVYVTPGSGQFMTTDDNQSP
2mev3         SPIPVTIREHAGTWYSTLPDSTV
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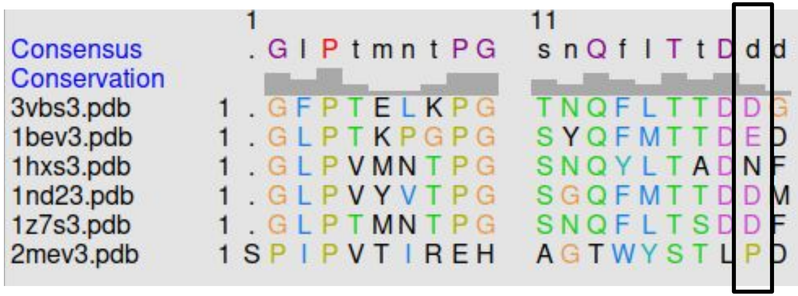
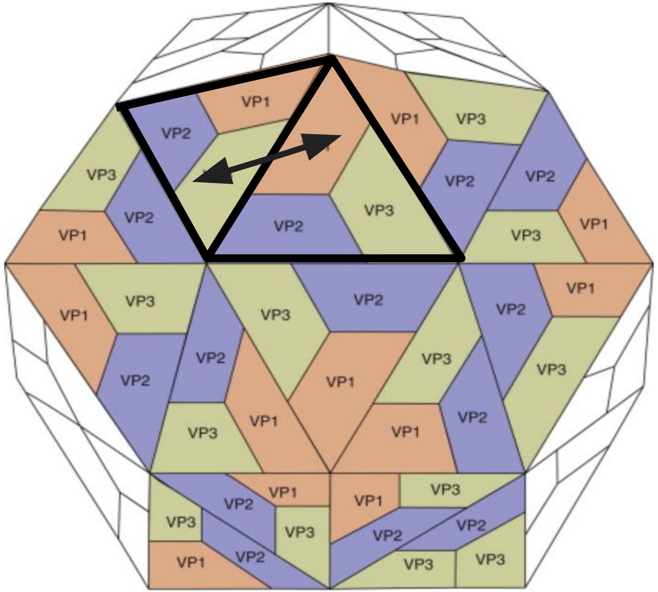
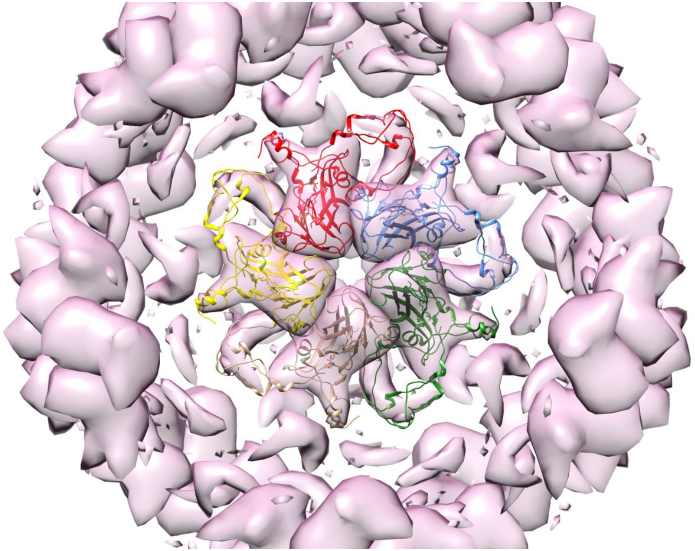


```
                  1                        11
Consensus        . G I P t m n t P G      s n Q f I T t D d d
Conservation
3vbs3.pdb     1  . G F P T E L K P G      T N Q F L T T D D G
1bev3.pdb     1  . G L P T K P G P G      S Y Q F M T T D E D
1hxs3.pdb     1  . G L P V M N T P G      S N Q Y L T A D N F
1nd23.pdb     1  . G L P V Y V T P G      S G Q F M T T D D M
1z7s3.pdb     1  . G L P T M N T P G      S N Q F L T S D D F
2mev3.pdb     1  S P I P V T I R E H      A G T W Y S T L P D
```

INTERPROTOMERIC INTERACTIONS

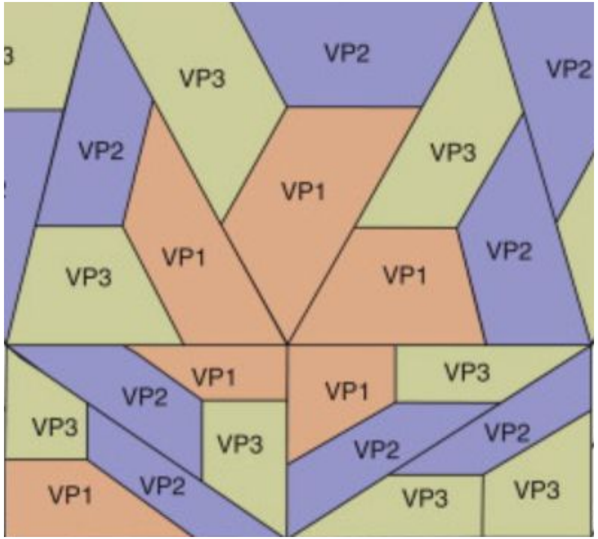


Interprotomeric Hydrogen Bonds VP1-VP1



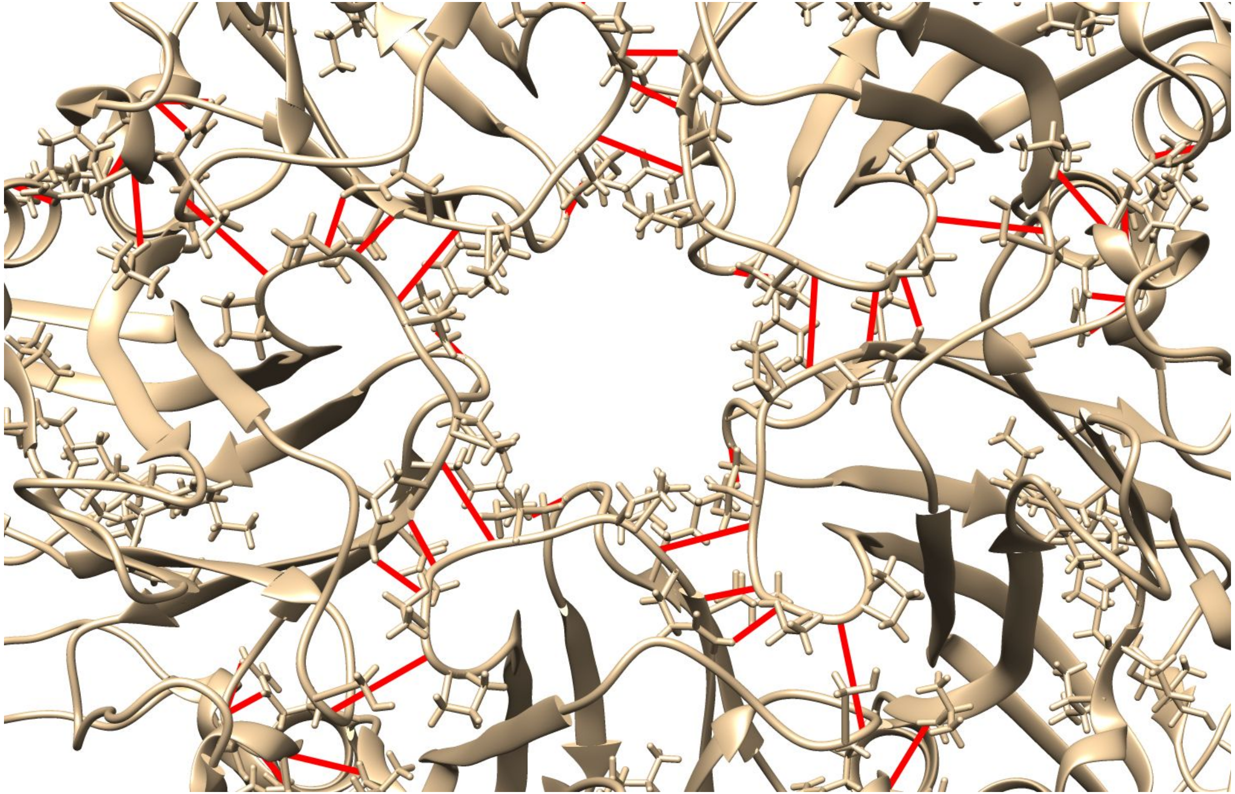
Only VP1s displayed

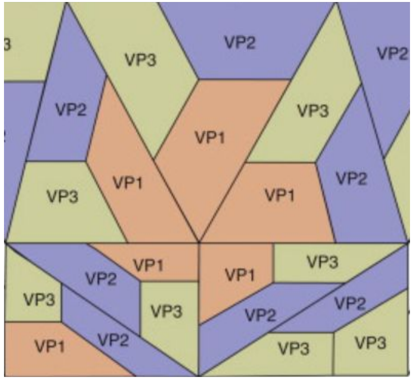
Each VP1 interacts with 2 neighbouring VP1s



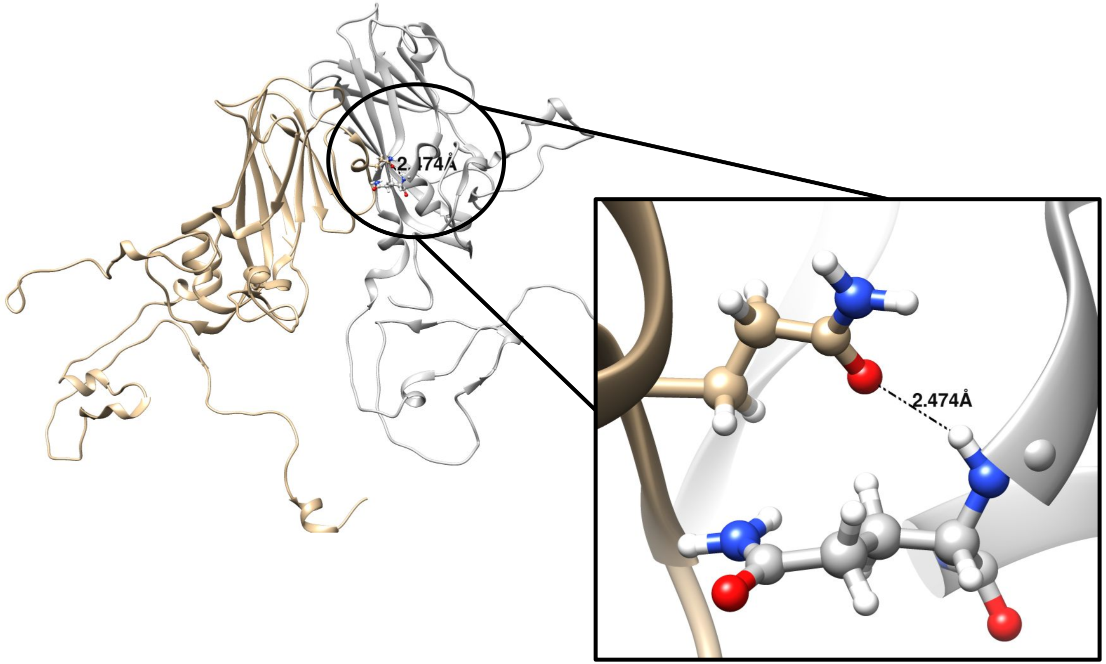
VP1s around 5-fold axis

Interprotomeric Hydrogen Bonds VP1-VP1

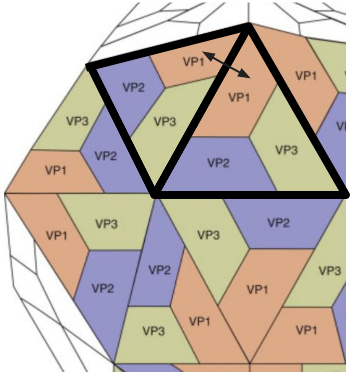




Interprotomeric Hydrogen Bonds VP1-VP1

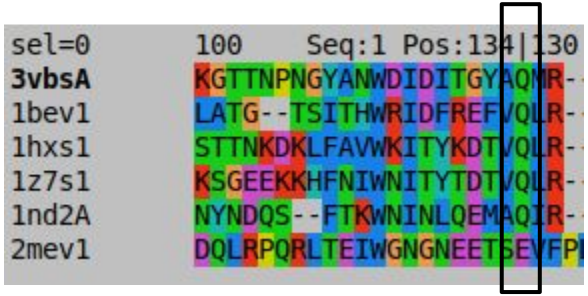


VP1	VP1	Distance (Å)
Glutamine 172 O Acceptor	Glutamine 118 NH Donor	2.474



Interprotomeric Hydrogen Bonds VP1-VP1

VP1 (Donor)



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sel=0        100    Seq:1 Pos:134|130
3vbsA        KGTTNPNGYANWDIDITGYAQMR--
1bev1        LATG--TSITHWRIDFREFVQLR--
1hxs1        STTNKDKLFAVWKITYKDTVQLR--
1z7s1        KSGEEKKHFNIWNITYTDTVQLR--
1nd2A        NYNDQS--FTKWNINLQEMAQIR--
2mev1        DQLRPQRLTEIWGNGNEETSEVFP
```
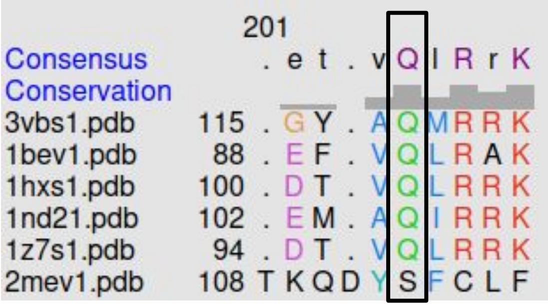
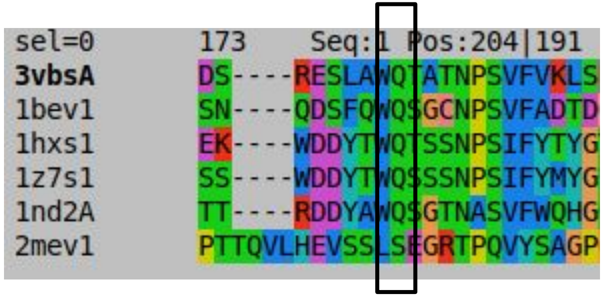
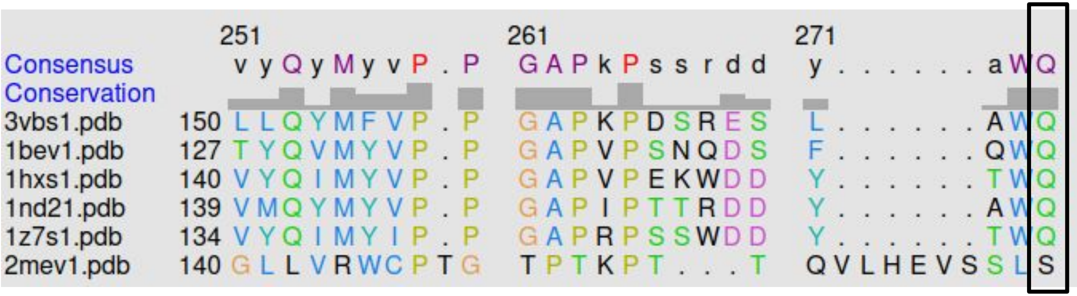



```
                    201
Consensus           . e t . v Q I R r K
Conservation
3vbs1.pdb    115    . G Y . A Q M R R K
1bev1.pdb     88    . E F . V Q L R A K
1hxs1.pdb    100    . D T . V Q L R R K
1nd21.pdb    102    . E M . A Q I R R K
1z7s1.pdb     94    . D T . V Q L R R K
2mev1.pdb    108    T K Q D Y S F C L F
```

VP1 (Acceptor)

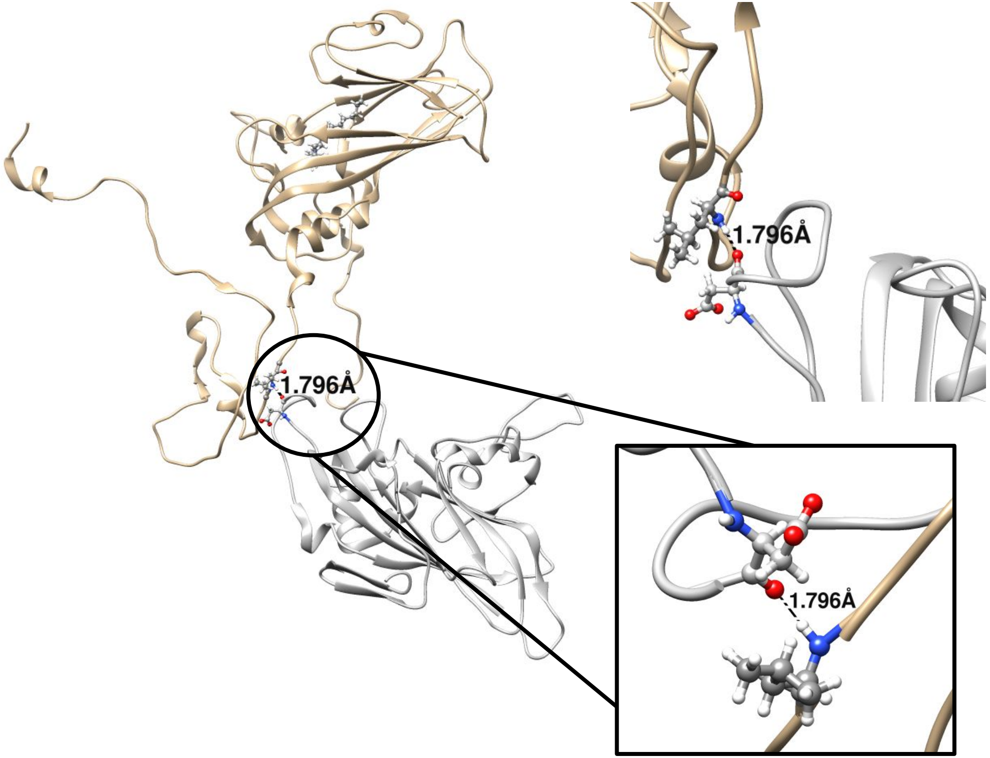


```
sel=0        173    Seq:1 Pos:204|191
3vbsA        DS----RESLAWQTATNPSVFVKLS
1bev1        SN----QDSFQWQSGCNPSVFADTD
1hxs1        EK----WDDYTWQTSSNPSIFYTYG
1z7s1        SS----WDDYTWQSSSNPSIFYMYG
1nd2A        TT----RDDYAWQSGTNASVFWQHG
2mev1        PTTQVLHEVSSLSEGRTPQVYSAGP
```

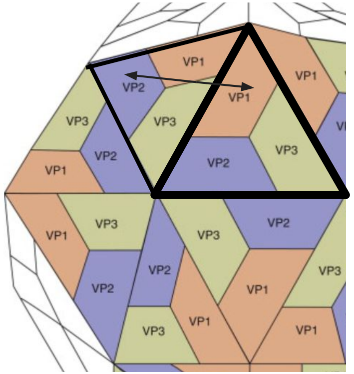


```
                   251                   261                   271
Consensus          v y Q y M y v P . P   G A P k P s s r d d   y . . . . . . . a W Q
Conservation
3vbs1.pdb    150   L L Q Y M F V P . P   G A P K P D S R E S   L . . . . . . . A W Q
1bev1.pdb    127   T Y Q V M Y V P . P   G A P V P S N Q D S   F . . . . . . . Q W Q
1hxs1.pdb    140   V Y Q I M Y V P . P   G A P V P E K W D D   Y . . . . . . . T W Q
1nd21.pdb    139   V M Q Y M Y V P . P   G A P I P T T R D D   Y . . . . . . . A W Q
1z7s1.pdb    134   V Y Q I M Y I P . P   G A P R P S S W D D   Y . . . . . . . T W Q
2mev1.pdb    140   G L L V R W C P T G   T P T K P T . . . T   Q V L H E V S S L S
```

Interprotomeric Hydrogen Bonds VP1-VP2

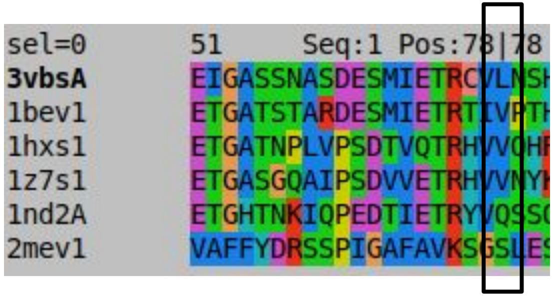


VP1	VP2	Distance (Å)
Leucine 70 NH Donor	Aspartate 44 O Acceptor	1.796



Interprotomeric Hydrogen Bonds VP1-VP2

VP1 (Donor)



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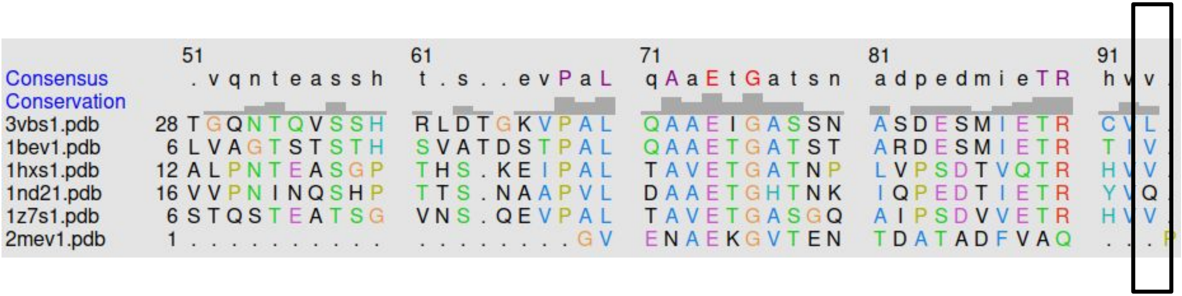
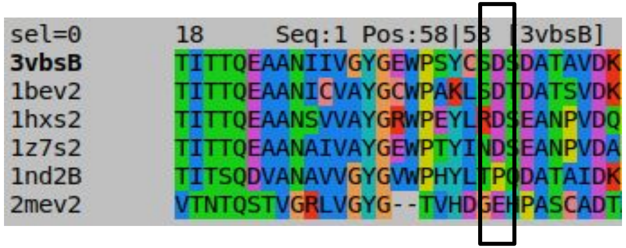
sel=0        51      Seq:1 Pos:78|78
3vbsA        EIGASSNASDESMIETRCVLNSH
1bev1        ETGATSTARDESMIETRTIVPTH
1hxs1        ETGATNPLVPSDTVQTRHVVQH
1z7s1        ETGASGQAIPSDVVETRHVVNY
1nd2A        ETGHTNKIQPEDTIETRYVQSS
2mev1        VAFFYDRSSPIGAFAVKSGSLE
```

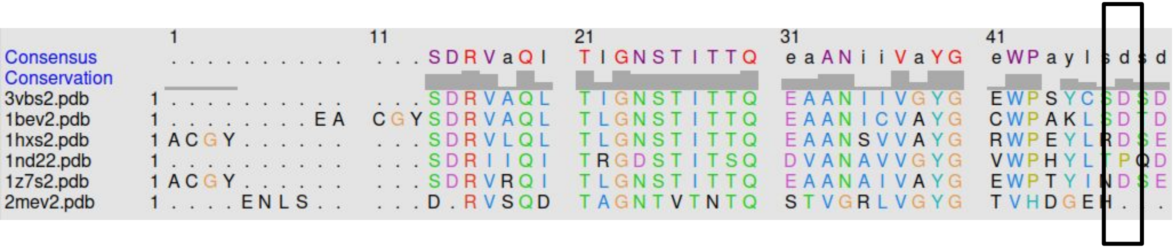


```
                   51                    61                    71                    81                    91
Consensus       . v q n t e a s s h   t . s . . e v P a L   q A a E t G a t s n   a d p e d m i e T R   h v v .
Conservation
3vbs1.pdb    28 T G Q N T Q V S S H   R L D T G K V P A L   Q A A E I G A S S N   A S D E S M I E T R   C V L .
1bev1.pdb     6 L V A G T S T S T H   S V A T D S T P A L   Q A A E T G A T S T   A R D E S M I E T R   T I V .
1hxs1.pdb    12 A L P N T E A S G P   T H S . K E I P A L   T A V E T G A T N P   L V P S D T V Q T R   H V V .
1nd21.pdb    16 V V P N I N Q S H P   T T S . N A A P V L   D A A E T G H T N K   I Q P E D T I E T R   Y V Q .
1z7s1.pdb     6 S T Q S T E A T S G   V N S . Q E V P A L   T A V E T G A S G Q   A I P S D V V E T R   H V V .
2mev1.pdb     1 . . . . . . . . . .   . . . . . . . . G V   E N A E K G V T E N   T D A T A D F V A Q   . . . P
```

VP2 (Acceptor)

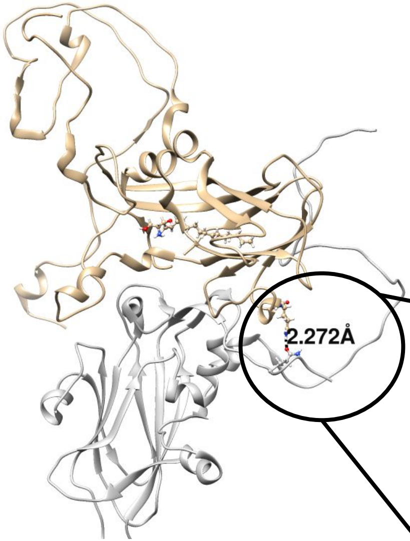


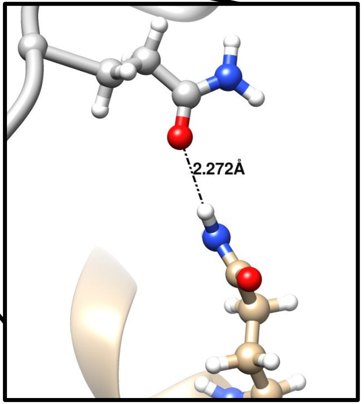
```
sel=0     18      Seq:1 Pos:58|58 [3vbsB]
3vbsB     TITTQEAANIIVGYGEWPSYCSDSDATAVDK
1bev2     TITTQEAANICVAYGCWPAKLSDTDATSVDK
1hxs2     TITTQEAANSVVAYGRWPEYLRDSEANPVDQ
1z7s2     TITTQEAANAIVAYGEWPTYINDSEANPVDA
1nd2B     TITSQDVANAVVGYGVWPHYLTPQDATAIDK
2mev2     VTNTQSTVGRLVGYG--TVHDGEHPASCADT
```

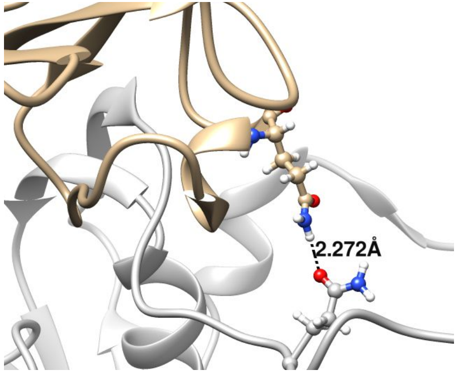


```
                 1                     11                    21                    31                    41
Consensus       . . . . . . . . . .   . . . S D R V a Q I   T I G N S T I T T Q   e a A N i i V a Y G   e W P a y l s d s d
Conservation
3vbs2.pdb     1 . . . . . . . . . .   . . . S D R V A Q L   T I G N S T I T T Q   E A A N I I V G Y G   E W P S Y C S D S D
1bev2.pdb     1 . . . . . . . . E A   C G Y S D R V A Q L   T L G N S T I T T Q   E A A N I C V A Y G   C W P A K L S D T D
1hxs2.pdb     1 A C G Y . . . . . .   . . . S D R V L Q L   T L G N S T I T T Q   E A A N S V V A Y G   R W P E Y L R D S E
1nd22.pdb     1 . . . . . . . . . .   . . . S D R I I Q I   T R G D S T I T S Q   D V A N A V V G Y G   V W P H Y L T P Q D
1z7s2.pdb     1 A C G Y . . . . . .   . . . S D R V R Q I   T L G N S T I T T Q   E A A N A I V A Y G   E W P T Y I N D S E
2mev2.pdb     1 . . . . E N L S . .   . . . D . R V S Q D   T A G N T V T N T Q   S T V G R L V G Y G   T V H D G E H . . .
```

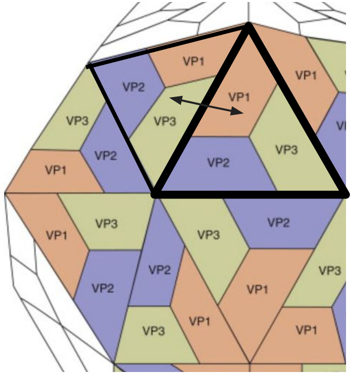
Interprotomeric Hydrogen Bonds VP1-VP3





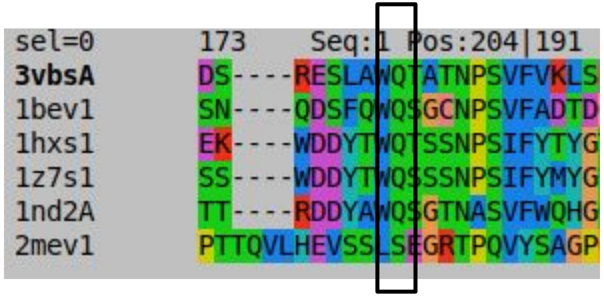


VP1	VP3	Distance (Å)
Glutamine 172 NH Donor	Glutamine 237 O Acceptor	2.272

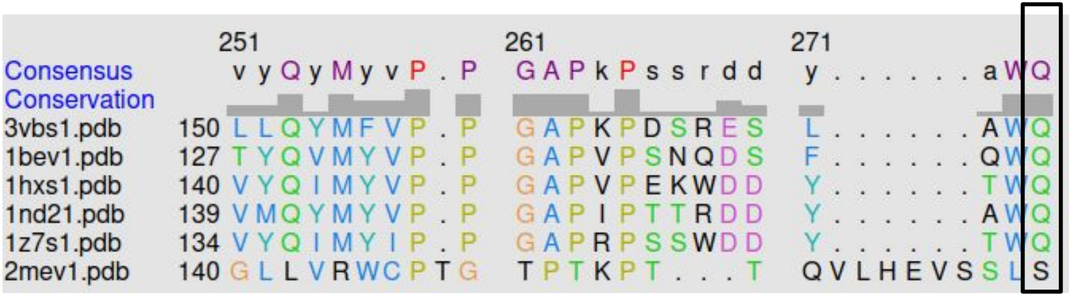


Interprotomeric Hydrogen Bonds VP1-VP3

VP1 (Donor)



```
sel=0       173     Seq:1 Pos:204|191
3vbsA       DS----RESLAWQTATNPSVFVKLS
1bev1       SN----QDSFQWQSGCNPSVFADTD
1hxs1       EK----WDDYTWQTSSNPSIFYTYG
1z7s1       SS----WDDYTWQSSSNPSIFYMYG
1nd2A       TT----RDDYAWQSGTNASVFWQHG
2mev1       PTTQVLHEVSSLSEGRTPQVYSAGP
```



```
                      251                   261                   271
Consensus             v y Q y M y v P . P   G A P k P s s r d d   y . . . . . . . a W Q
Conservation
3vbs1.pdb       150   L L Q Y M F V P . P   G A P K P D S R E S   L . . . . . . . A W Q
1bev1.pdb       127   T Y Q V M Y V P . P   G A P V P S N Q D S   F . . . . . . . Q W Q
1hxs1.pdb       140   V Y Q I M Y V P . P   G A P V P E K W D D   Y . . . . . . . T W Q
1nd21.pdb       139   V M Q Y M Y V P . P   G A P I P T T R D D   Y . . . . . . . A W Q
1z7s1.pdb       134   V Y Q I M Y I P . P   G A P R P S S W D D   Y . . . . . . . T W Q
2mev1.pdb       140   G L L V R W C P T G   T P T K P T . . . T   Q V L H E V S S L S
```

VP3 (Acceptor)

```
sel=0
3vbsC       ALAAAQKNFTMKLCKDASDILQTGTIQ
1bev3       MMIAAQPNFSFRIQKDREDMTQTAILQ
1hxs3       GFVSACNDFSVRLLRDTTHIEQKA---
1z7s3       GFVSACPDFSVRLLKDTPHISQSKLIG
1nd2C       CFVSGCKDFCLRMARDTDLHKQTGPIT
2mev3       TMVSAGKDFSLKMPISPAPWSPQ----
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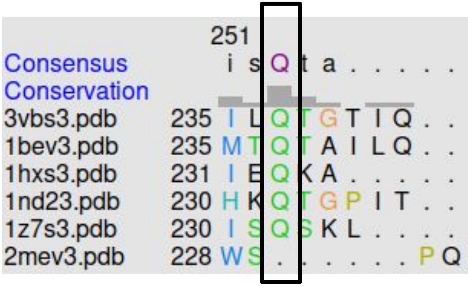
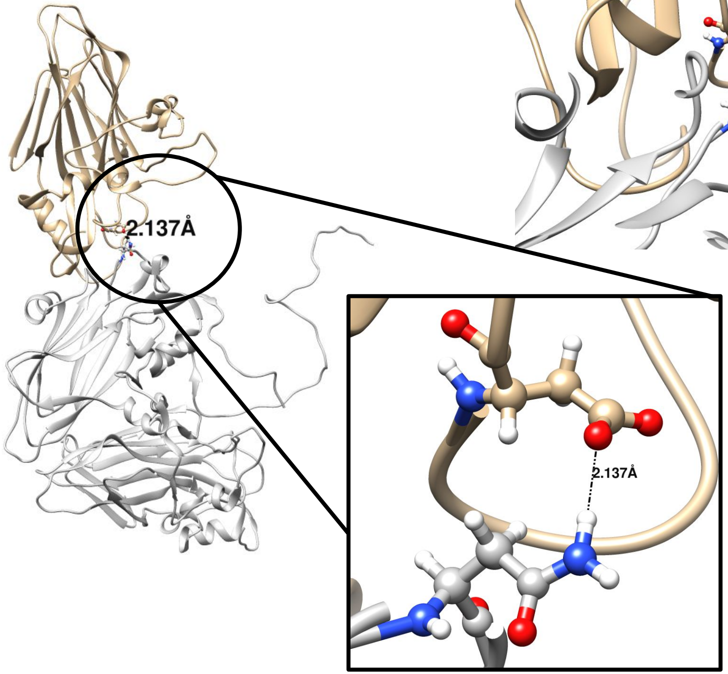
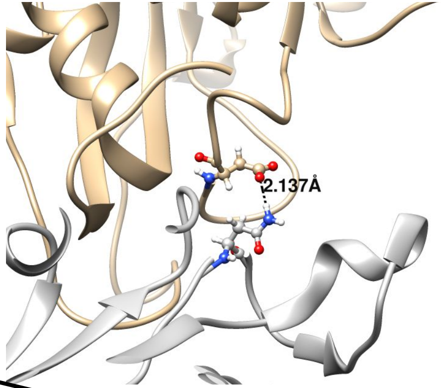


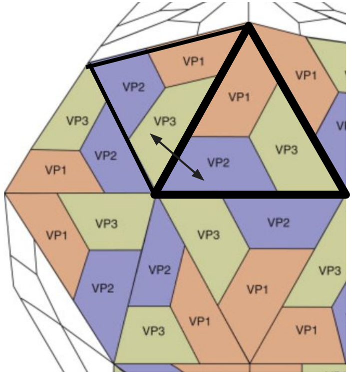
```
                       251
Consensus              i s Q t a . . . . .
Conservation
3vbs3.pdb       235    I L Q T G T I Q . .
1bev3.pdb       235    M T Q T A I L Q . .
1hxs3.pdb       231    I E Q K A . . . . .
1nd23.pdb       230    H K Q T G P I T . .
1z7s3.pdb       230    I S Q S K L . . . .
2mev3.pdb       228    W S . . . . . . P Q
```

Interprotomeric Hydrogen Bonds VP2-VP3



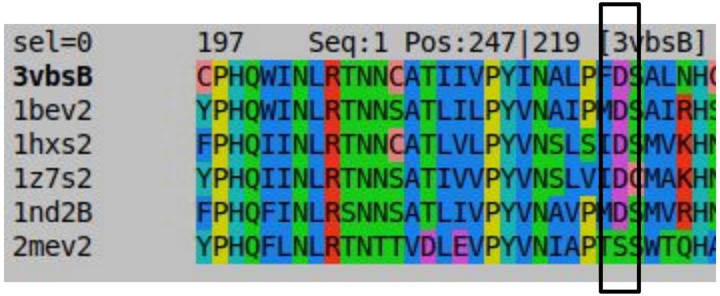


VP2	VP3	Distance (Å)
Aspartate 195 O Acceptor	Asparagine 174 NH Donor	2.137

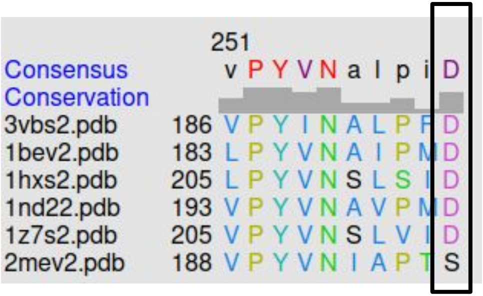


Interprotomeric Hydrogen Bonds VP2-VP3

VP2 (Acceptor)

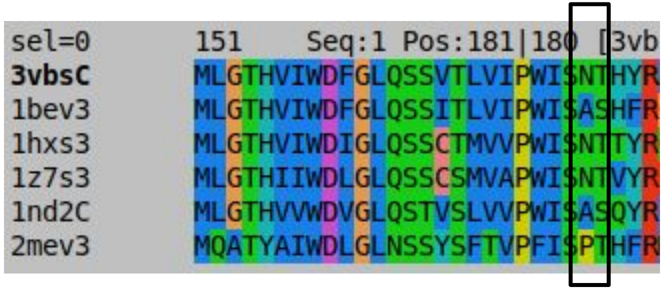


```
sel=0        197     Seq:1 Pos:247|219 [3vbsB]
3vbsB        CPHQWINLRTNNCATIIVPYINALPFDSALNH
1bev2        YPHQWINLRTNNSATLILPYVNAIPMDSAIRH
1hxs2        FPHQIINLRTNNCATLVLPYVNSLSIDSMVKH
1z7s2        YPHQIINLRTNNSATIVVPYVNSLVIDCMAKH
1nd2B        FPHQFINLRSNNSATLIVPYVNAVPMDSMVRH
2mev2        YPHQFLNLRTNTTVDLEVPYVNIAPTSSWTQH
```

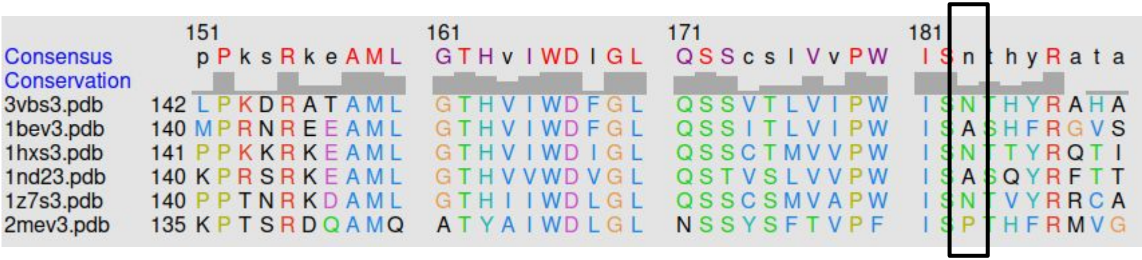


```
                   251
Consensus          v P Y V N a l p i D
Conservation
3vbs2.pdb     186  V P Y I N A L P F D
1bev2.pdb     183  L P Y V N A I P M D
1hxs2.pdb     205  L P Y V N S L S I D
1nd22.pdb     193  V P Y V N A V P M D
1z7s2.pdb     205  V P Y V N S L V I D
2mev2.pdb     188  V P Y V N I A P T S
```

VP3 (Donor)

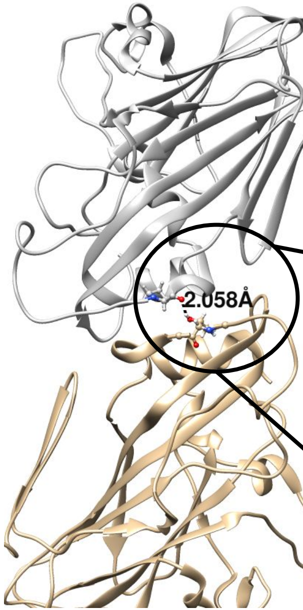


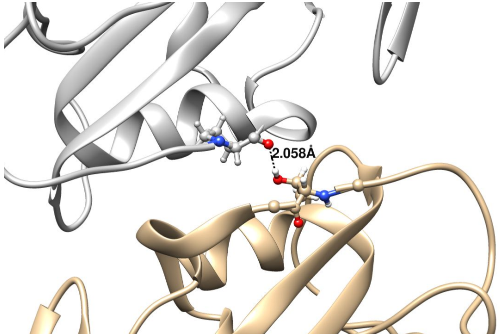
```
sel=0        151     Seq:1 Pos:181|180 [3vb
3vbsC        MLGTHVIWDFGLQSSVTLVIPWISNTHYR
1bev3        MLGTHVIWDFGLQSSITLVIPWISASHFR
1hxs3        MLGTHVIWDIGLQSSCTMVVPWISNTTYR
1z7s3        MLGTHIIWDLGLQSSCSMVAPWISNTVYR
1nd2C        MLGTHVVWDVGLQSTVSLVVPWISASQYR
2mev3        MQATYAIWDLGLNSSYSFTVPFISPTHFR
```

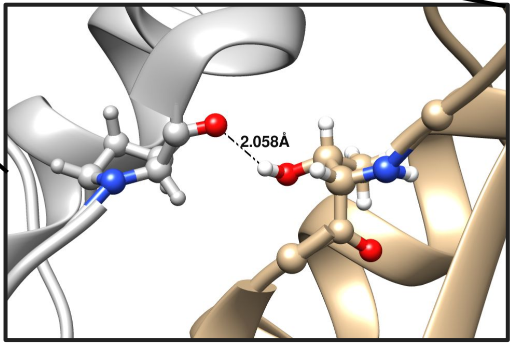


```
                   151                  161                  171                  181
Consensus          p P k s R k e A M L  G T H v I W D I G L  Q S S c s I V v P W  I S n t h y R a t a
Conservation
3vbs3.pdb     142  L P K D R A T A M L  G T H V I W D F G L  Q S S V T L V I P W  I S N T H Y R A H A
1bev3.pdb     140  M P R N R E E A M L  G T H V I W D F G L  Q S S I T L V I P W  I S A S H F R G V S
1hxs3.pdb     141  P P K K R K E A M L  G T H V I W D I G L  Q S S C T M V V P W  I S N T T Y R Q T I
1nd23.pdb     140  K P R S R K E A M L  G T H V V W D V G L  Q S T V S L V V P W  I S A S Q Y R F T T
1z7s3.pdb     140  P P T N R K D A M L  G T H I I W D L G L  Q S S C S M V A P W  I S N T V Y R R C A
2mev3.pdb     135  K P T S R D Q A M Q  A T Y A I W D L G L  N S S Y S F T V P F  I S P T H F R M V G
```

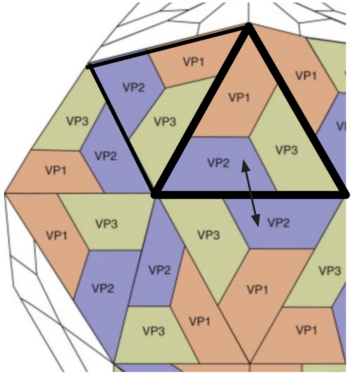
Interprotomeric Hydrogen Bonds VP2-VP2





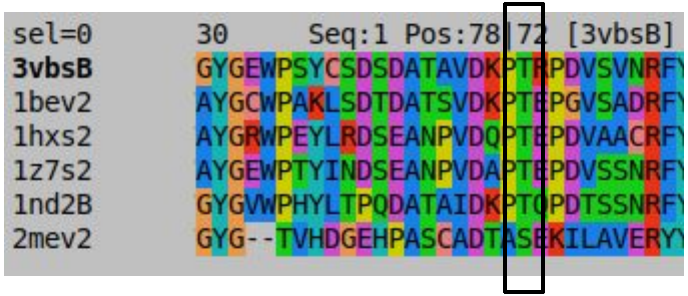


VP2	VP2	Distance (Å)
Threonine 45 OH Donor	Proline 47 O Acceptor	2.058

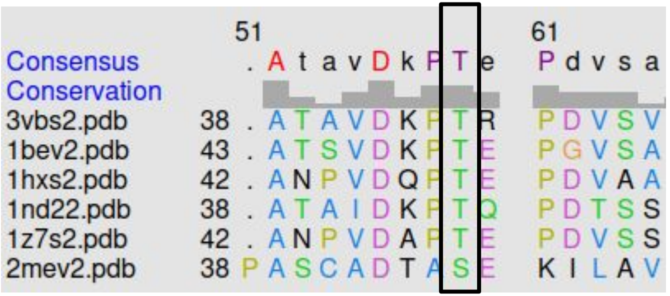


Interprotomeric Hydrogen Bonds VP2-VP2

VP2 (Donor)

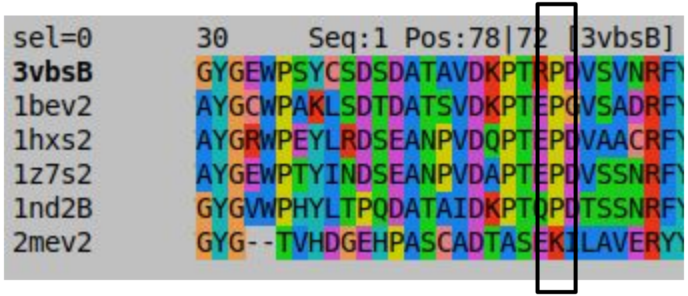


```
sel=0         30     Seq:1 Pos:78|72 [3vbsB]
3vbsB         GYGEWPSYCSDSDATAVDKPTRPDVSVNRFY
1bev2         AYGCWPAKLSDTDATSVDKPTEPGVSADRFY
1hxs2         AYGRWPEYLRDSEANPVDQPTEPDVAACRFY
1z7s2         AYGEWPTYINDSEANPVDAPTEPDVSSNRFY
1nd2B         GYGVWPHYLTPQDATAIDKPTQPDTSSNRFY
2mev2         GYG--TVHDGEHPASCADTASEKILAVERYY
```

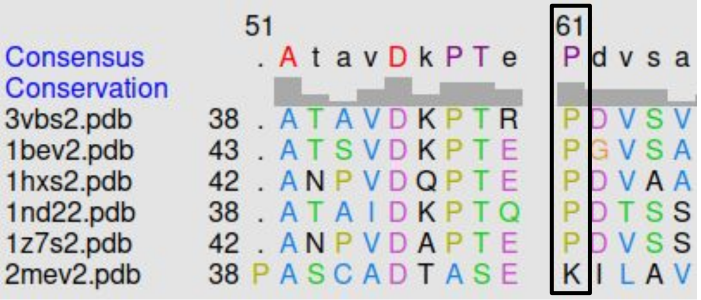


```
                   51                 61
Consensus        . A t a v D k P T e  P d v s a
Conservation
3vbs2.pdb     38 . A T A V D K P T R  P D V S V
1bev2.pdb     43 . A T S V D K P T E  P G V S A
1hxs2.pdb     42 . A N P V D Q P T E  P D V A A
1nd22.pdb     38 . A T A I D K P T Q  P D T S S
1z7s2.pdb     42 . A N P V D A P T E  P D V S S
2mev2.pdb     38 P A S C A D T A S E  K I L A V
```

VP2 (Acceptor)

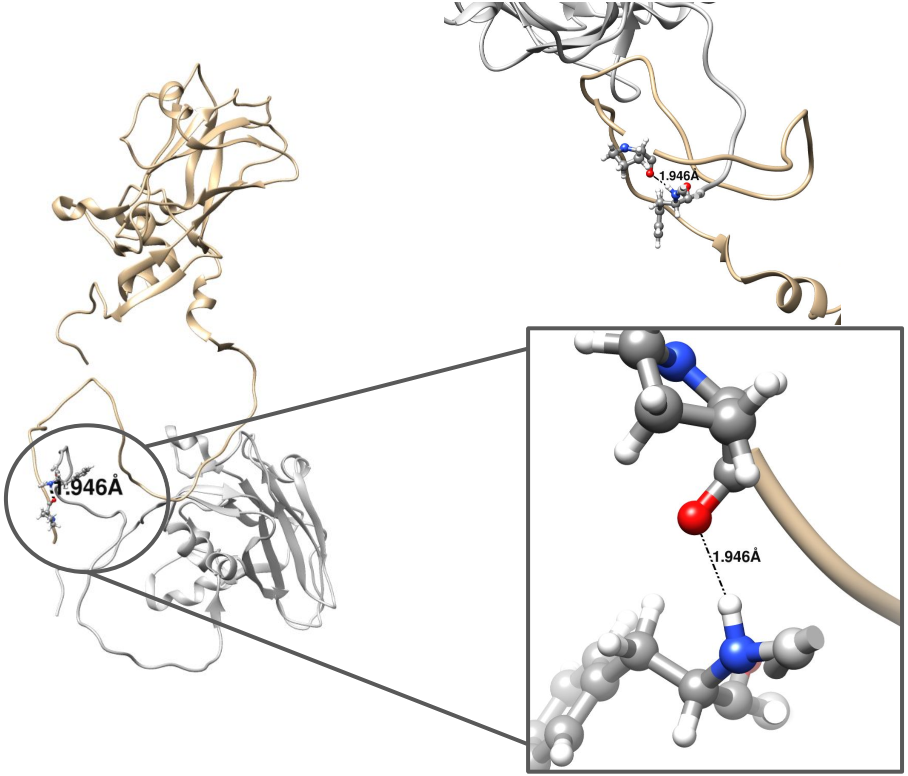


```
sel=0         30     Seq:1 Pos:78|72 [3vbsB]
3vbsB         GYGEWPSYCSDSDATAVDKPTRPDVSVNRFY
1bev2         AYGCWPAKLSDTDATSVDKPTEPGVSADRFY
1hxs2         AYGRWPEYLRDSEANPVDQPTEPDVAACRFY
1z7s2         AYGEWPTYINDSEANPVDAPTEPDVSSNRFY
1nd2B         GYGVWPHYLTPQDATAIDKPTQPDTSSNRFY
2mev2         GYG--TVHDGEHPASCADTASEKILAVERYY
```

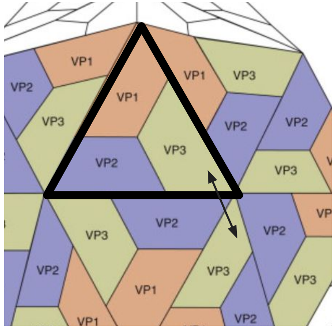


```
                   51                 61
Consensus        . A t a v D k P T e  P d v s a
Conservation
3vbs2.pdb     38 . A T A V D K P T R  P D V S V
1bev2.pdb     43 . A T S V D K P T E  P G V S A
1hxs2.pdb     42 . A N P V D Q P T E  P D V A A
1nd22.pdb     38 . A T A I D K P T Q  P D T S S
1z7s2.pdb     42 . A N P V D A P T E  P D V S S
2mev2.pdb     38 P A S C A D T A S E  K I L A V
```

Interprotomeric Hydrogen Bonds VP3-VP3

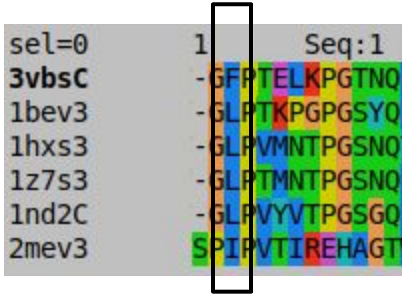


VP3	VP3	Distance (Å)
Proline 3 O Acceptor	Phenylalanine 2 NH Donor	1.946



Interprotomeric Hydrogen Bonds VP3-VP3

VP3 (Donor)



sel=0 1 Seq:1

3vbsC -GFPTELKPGTNQ

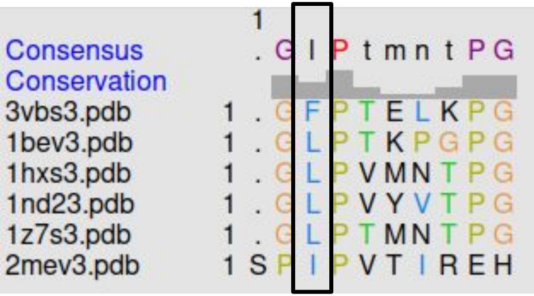
1bev3 -GLPTKPGPGSYQ

1hxs3 -GLPVMNTPGSNQ

1z7s3 -GLPTMNTPGSNQ

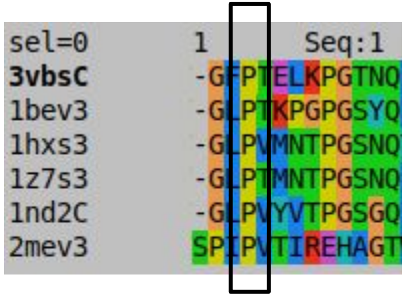
1nd2C -GLPVYVTPGSGQ

2mev3 SPIPVTIREHAGT



		1
Consensus		. G I P t m n t P G
Conservation		
3vbs3.pdb	1	. G F P T E L K P G
1bev3.pdb	1	. G L P T K P G P G
1hxs3.pdb	1	. G L P V M N T P G
1nd23.pdb	1	. G L P V Y V T P G
1z7s3.pdb	1	. G L P T M N T P G
2mev3.pdb	1	S P I P V T I R E H

VP3 (Acceptor)



sel=0 1 Seq:1

3vbsC -GFPTELKPGTNQ

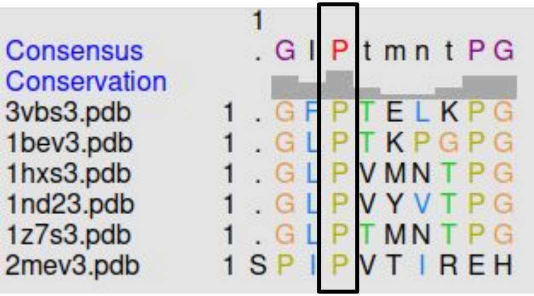
1bev3 -GLPTKPGPGSYQ

1hxs3 -GLPVMNTPGSNQ

1z7s3 -GLPTMNTPGSNQ

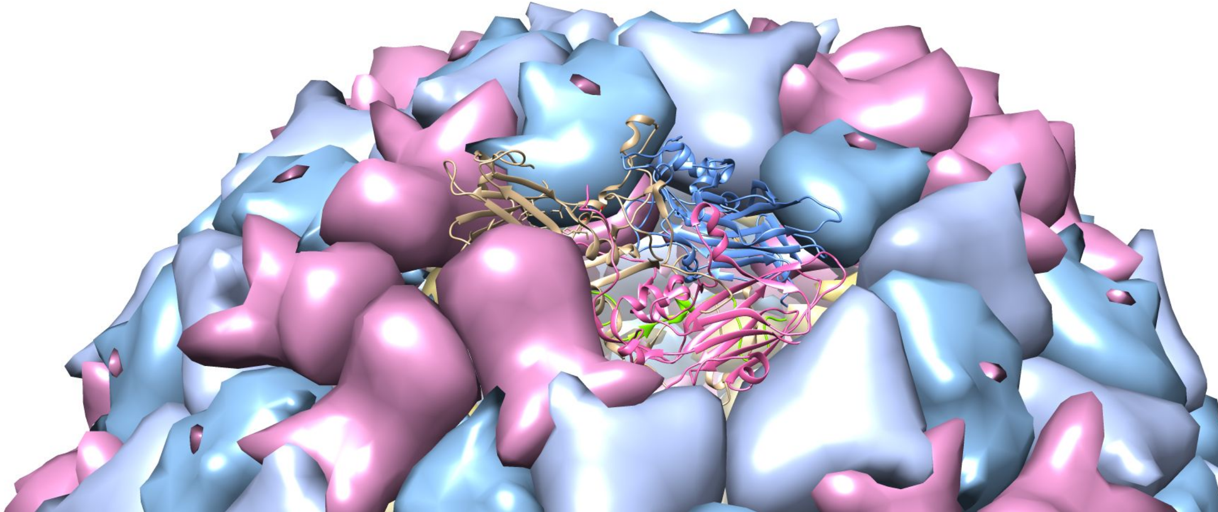
1nd2C -GLPVYVTPGSGQ

2mev3 SPIPVTIREHAGT



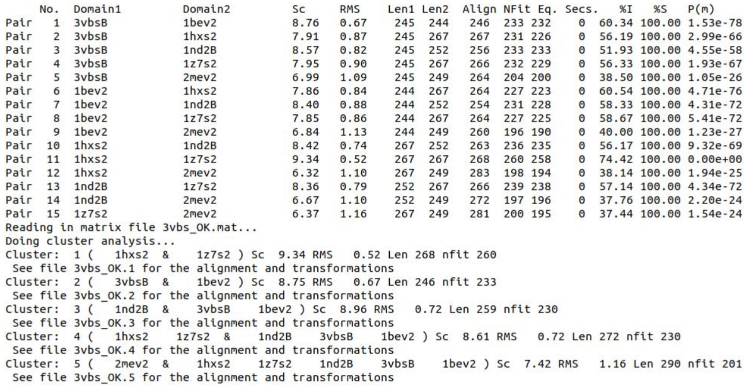
		1
Consensus		. G I P t m n t P G
Conservation		
3vbs3.pdb	1	. G F P T E L K P G
1bev3.pdb	1	. G L P T K P G P G
1hxs3.pdb	1	. G L P V M N T P G
1nd23.pdb	1	. G L P V Y V T P G
1z7s3.pdb	1	. G L P T M N T P G
2mev3.pdb	1	S P I P V T I R E H

3. Sequence and structural comparison along evolution



Methodology

1. We selected our templates after a **PSI-BLAST**
2. We performed a **CLUSTALW** alignment
3. We performed a **STAMP** alignment (*roughfit* gave many LOW SCORES warnings, so we used the *alignfit* option)
4. We analyzed the clusters formed and created a **phylogenetic tree**



```
     No.  Domain1          Domain2          Sc      RMS     Len1 Len2  Align NFit Eq. Secs.   %I     %S    P(m)
Pair   1  3vbsB            1bev2            8.76    0.67     245  244    246  233 232     0  60.34 100.00 1.53e-78
Pair   2  3vbsB            1hxs2            7.91    0.87     245  267    267  231 226     0  56.19 100.00 2.99e-66
Pair   3  3vbsB            1nd2B            8.57    0.82     245  252    256  233 233     0  51.93 100.00 4.55e-58
Pair   4  3vbsB            1z7s2            7.95    0.90     245  267    266  232 229     0  56.33 100.00 1.93e-67
Pair   5  3vbsB            2mev2            6.99    1.09     245  249    264  204 200     0  38.50 100.00 1.05e-26
Pair   6  1bev2            1hxs2            7.86    0.84     244  267    264  227 223     0  60.54 100.00 4.71e-76
Pair   7  1bev2            1nd2B            8.40    0.88     244  252    254  231 228     0  58.33 100.00 4.31e-72
Pair   8  1bev2            1z7s2            7.85    0.86     244  267    264  227 225     0  58.67 100.00 5.41e-72
Pair   9  1bev2            2mev2            6.84    1.13     244  249    260  196 190     0  40.00 100.00 1.23e-27
Pair  10  1hxs2            1nd2B            8.42    0.74     267  252    263  236 235     0  56.17 100.00 9.32e-69
Pair  11  1hxs2            1z7s2            9.34    0.52     267  267    268  260 258     0  74.42 100.00 0.00e+00
Pair  12  1hxs2            2mev2            6.32    1.10     267  249    283  198 194     0  38.14 100.00 1.94e-25
Pair  13  1nd2B            1z7s2            8.36    0.79     252  267    266  239 238     0  57.14 100.00 4.34e-72
Pair  14  1nd2B            2mev2            6.67    1.10     252  249    272  197 196     0  37.76 100.00 2.20e-24
Pair  15  1z7s2            2mev2            6.37    1.16     267  249    281  200 195     0  37.44 100.00 1.54e-24
Reading in matrix file 3vbs_OK.mat...
Doing cluster analysis...
Cluster:  1 (   1hxs2  &    1z7s2 ) Sc  9.34 RMS   0.52 Len 268 nfit 260
 See file 3vbs_OK.1 for the alignment and transformations
Cluster:  2 (   3vbsB  &    1bev2 ) Sc  8.75 RMS   0.67 Len 246 nfit 233
 See file 3vbs_OK.2 for the alignment and transformations
Cluster:  3 (   1nd2B  &    3vbsB    1bev2 ) Sc  8.96 RMS   0.72 Len 259 nfit 230
 See file 3vbs_OK.3 for the alignment and transformations
Cluster:  4 (   1hxs2    1z7s2  &    1nd2B    3vbsB    1bev2 ) Sc  8.61 RMS   0.72 Len 272 nfit 230
 See file 3vbs_OK.4 for the alignment and transformations
Cluster:  5 (   2mev2  &    1hxs2    1z7s2    1nd2B    3vbsB    1bev2 ) Sc  7.42 RMS   1.16 Len 290 nfit 201
 See file 3vbs_OK.5 for the alignment and transformations
```

Example of STAMP output

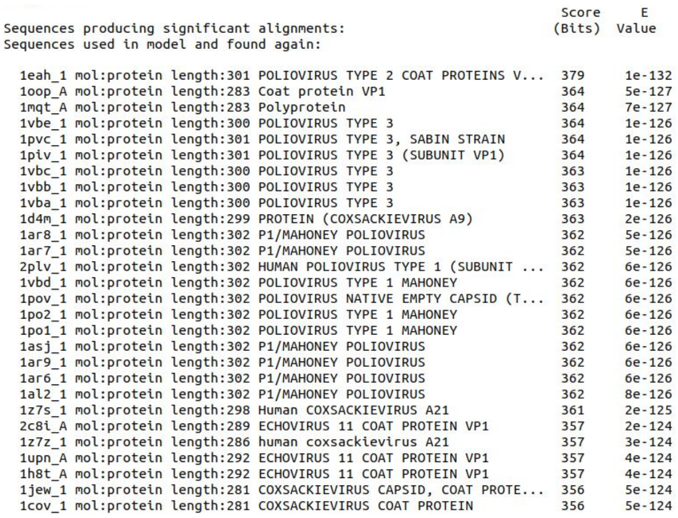
Reminder: what does my Sc and RMSD mean?

- 5.5 to 9.8 = high degree of structural similarity + functional and/or evolutionary relationship
- 2.5 to 5.5 = more distantly related structures + not always implies a functional or evolutionary relationship
- < 2.0 = little overall structural similarity

RMSD increases as similarity decreases

Template Selection

- Database used: PDB
- Selected templates from round 5
- We checked that the templates were a hit for all VPs
- We split the chains for every template



```
                                                                      Score     E
Sequences producing significant alignments:                          (Bits)  Value
Sequences used in model and found again:

  1eah_1 mol:protein length:301 POLIOVIRUS TYPE 2 COAT PROTEINS V...  379     1e-132
  1oop_A mol:protein length:283 Coat protein VP1                      364     5e-127
  1mqt_A mol:protein length:283 Polyprotein                           364     7e-127
  1vbe_1 mol:protein length:300 POLIOVIRUS TYPE 3                     364     1e-126
  1pvc_1 mol:protein length:301 POLIOVIRUS TYPE 3, SABIN STRAIN       364     1e-126
  1piv_1 mol:protein length:301 POLIOVIRUS TYPE 3 (SUBUNIT VP1)       364     1e-126
  1vbc_1 mol:protein length:300 POLIOVIRUS TYPE 3                     363     1e-126
  1vbb_1 mol:protein length:300 POLIOVIRUS TYPE 3                     363     1e-126
  1vba_1 mol:protein length:300 POLIOVIRUS TYPE 3                     363     1e-126
  1d4m_1 mol:protein length:299 PROTEIN (COXSACKIEVIRUS A9)           363     2e-126
  1ar8_1 mol:protein length:302 P1/MAHONEY POLIOVIRUS                 362     5e-126
  1ar7_1 mol:protein length:302 P1/MAHONEY POLIOVIRUS                 362     5e-126
  2plv_1 mol:protein length:302 HUMAN POLIOVIRUS TYPE 1 (SUBUNIT ...  362     6e-126
  1vbd_1 mol:protein length:302 POLIOVIRUS TYPE 1 MAHONEY             362     6e-126
  1pov_1 mol:protein length:302 POLIOVIRUS NATIVE EMPTY CAPSID (T...  362     6e-126
  1po2_1 mol:protein length:302 POLIOVIRUS TYPE 1 MAHONEY             362     6e-126
  1po1_1 mol:protein length:302 POLIOVIRUS TYPE 1 MAHONEY             362     6e-126
  1asj_1 mol:protein length:302 P1/MAHONEY POLIOVIRUS                 362     6e-126
  1ar9_1 mol:protein length:302 P1/MAHONEY POLIOVIRUS                 362     6e-126
  1ar6_1 mol:protein length:302 P1/MAHONEY POLIOVIRUS                 362     6e-126
  1al2_1 mol:protein length:302 P1/MAHONEY POLIOVIRUS                 362     8e-126
  1z7s_1 mol:protein length:298 Human COXSACKIEVIRUS A21              361     2e-125
  2c8i_A mol:protein length:289 ECHOVIRUS 11 COAT PROTEIN VP1         357     2e-124
  1z7z_1 mol:protein length:286 human coxsackievirus A21              357     3e-124
  1upn_A mol:protein length:292 ECHOVIRUS 11 COAT PROTEIN VP1         357     4e-124
  1h8t_A mol:protein length:292 ECHOVIRUS 11 COAT PROTEIN VP1         357     4e-124
  1jew_1 mol:protein length:281 COXSACKIEVIRUS CAPSID, COAT PROTE...  356     5e-124
  1cov_1 mol:protein length:281 COXSACKIEVIRUS COAT PROTEIN           356     5e-124
```

Part of the PSI-BLAST output

2mev = Mengo encephalomyelitis virus
1nd2 = Human rhinovirus 16
1hxs = Human Poliovirus 1 Mahoney
1z7s = Coxsackievirus A21
3vbs = Human Enterovirus 71
1bev = Bovine enterovirus strain VG-5-27

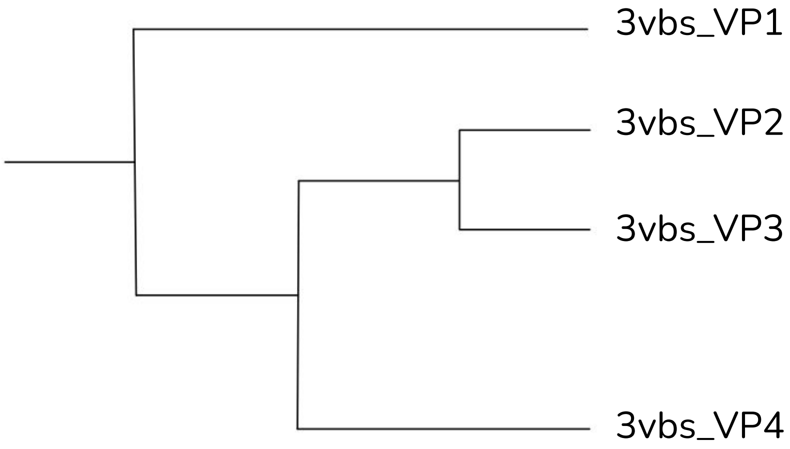
VS

2mev = Mengo encephalomyelitis virus
1nd2 = Human rhinovirus 16
1hxs = Human Poliovirus 1 Mahoney
1z7s = Coxsackievirus A21
3vbs = Human Enterovirus 71
1bev = Bovine enterovirus strain VG-5-27

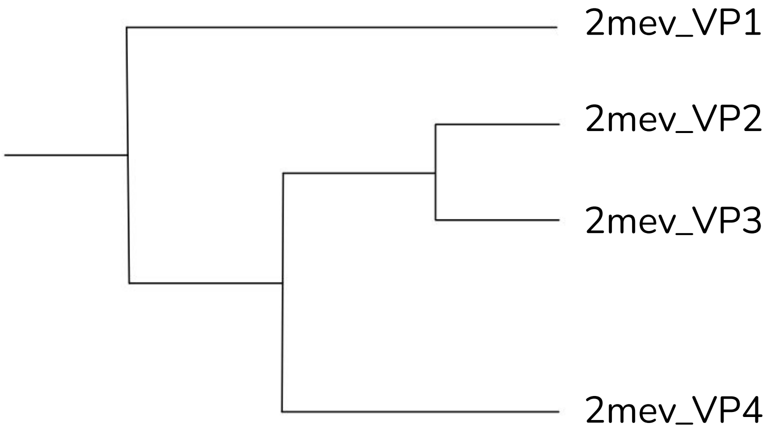
Enterovirus A, Enterovirus C, Enterovirus E, Rhinovirus A, Cardiovirus

Phylogeny of the capsid polyprotein

ENTEROVIRUS



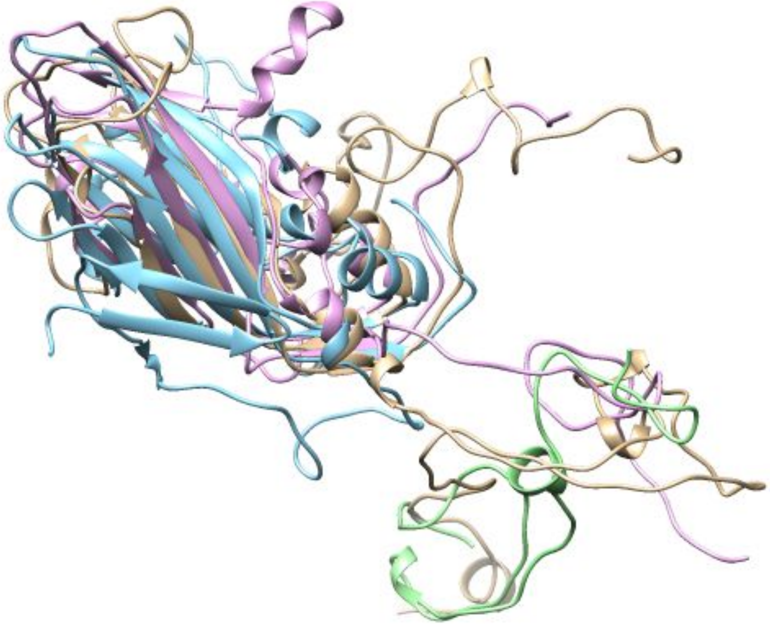
CARDIOVIRUS



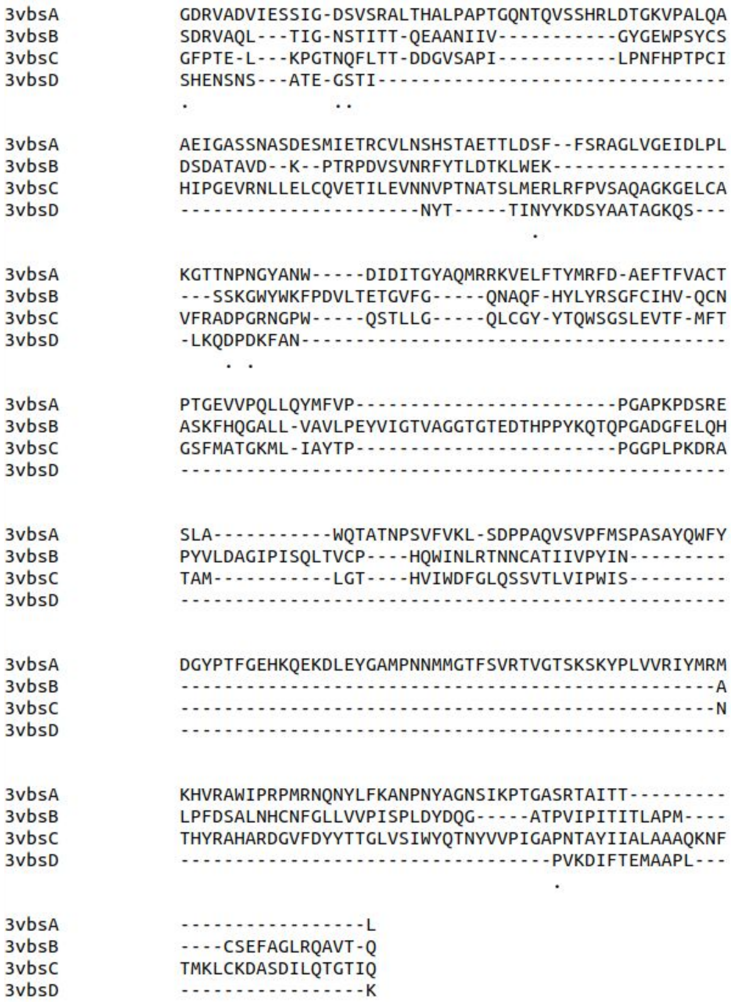
Common ancestor with 4 VP

VP1, VP2 and VP3 form monophyletic groups with similar inner topology

→ **2 paralogous duplication events** prior to the divergence of *Picornavirales*

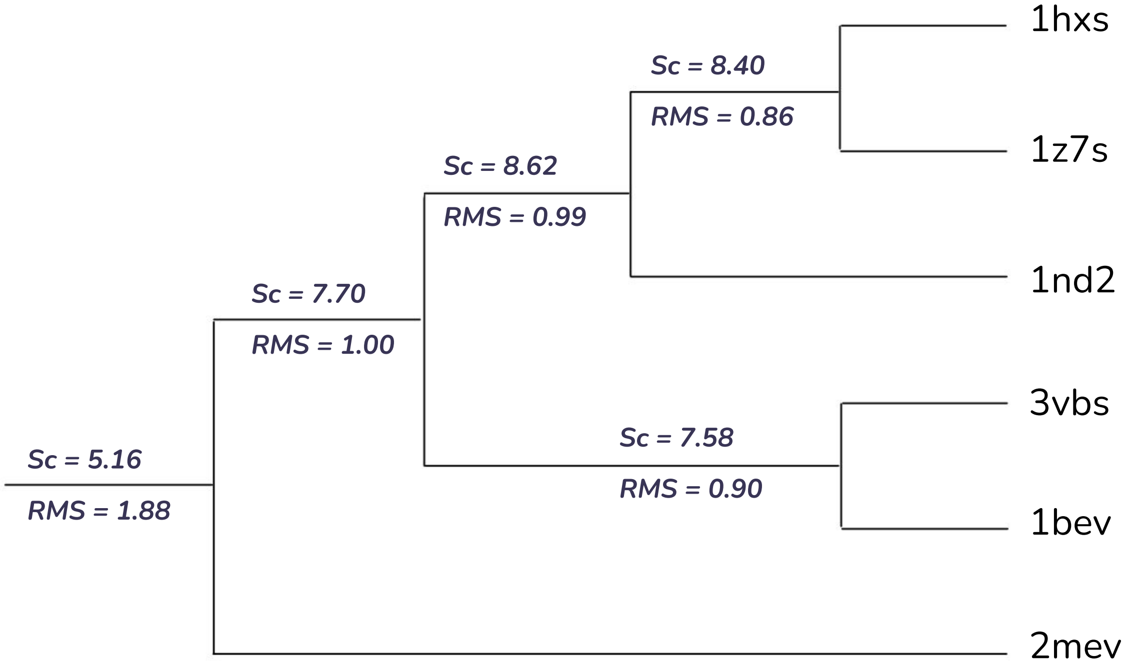


- VP1, VP2 and VP3 share the same topology despite not being much conserved between them, as for sequence
- VP4 is the most divergent of all the VP → STAMP gives LOW SCORE warnings



```
3vbsA          GDRVADVIESSIG-DSVSRALTHALPAPTGQNTQVSSHRLDTGKVPALQA
3vbsB          SDRVAQL---TIG-NSTITT-QEAANIIV-----------GYGEWPSYCS
3vbsC          GFPTE-L---KPGTNQFLTT-DDGVSAPI-----------LPNFHPTPCI
3vbsD          SHENSNS---ATE-GSTI--------------------------------
               .             ..

3vbsA          AEIGASSNASDESMIETRCVLNSHSTAETTLDSF--FSRAGLVGEIDLPL
3vbsB          DSDATAVD--K--PTRPDVSVNRFYTLDTKLWEK----------------
3vbsC          HIPGEVRNLLELCQVETILEVNNVPTNATSLMERLRFPVSAQAGKGELCA
3vbsD          ----------------------NYT-----TINYYKDSYAATAGKQS---
                                               .

3vbsA          KGTTNPNGYANW-----DIDITGYAQMRRKVELFTYMRFD-AEFTFVACT
3vbsB          ---SSKGWYWKFPDVLTETGVFG-----QNAQF-HYLYRSGFCIHV-QCN
3vbsC          VFRADPGRNGPW-----QSTLLG-----QLCGY-YTQWSGSLEVTF-MFT
3vbsD          -LKQDPDKFAN---------------------------------------
                   . .

3vbsA          PTGEVVPQLLQYMFVP------------------------PGAPKPDSRE
3vbsB          ASKFHQGALL-VAVLPEYVIGTVAGGTGTEDTHPPYKQTQPGADGFELQH
3vbsC          GSFMATGKML-IAYTP------------------------PGGPLPKDRA
3vbsD          --------------------------------------------------

3vbsA          SLA-----------WQTATNPSVFVKL-SDPPAQVSVPFMSPASAYQWFY
3vbsB          PYVLDAGIPISQLTVCP----HQWINLRTNNCATIIVPYIN---------
3vbsC          TAM-----------LGT----HVIWDFGLQSSVTLVIPWIS---------
3vbsD          --------------------------------------------------

3vbsA          DGYPTFGEHKQEKDLEYGAMPNNMMGTFSVRTVGTSKSKYPLVVRIYMRM
3vbsB          -------------------------------------------------A
3vbsC          -------------------------------------------------N
3vbsD          --------------------------------------------------

3vbsA          KHVRAWIPRPMRNQNYLFKANPNYAGNSIKPTGASRTAITT---------
3vbsB          LPFDSALNHCNFGLLVVPISPLDYDQG-----ATPVIPITITLAPM----
3vbsC          THYRAHARDGVFDYYTTGLVSIWYQTNYVVPIGAPNTAYIIALAAAQKNF
3vbsD          ----------------------------------PVKDIFTEMAAPL---
                                                 .

3vbsA          -----------------L
3vbsB          ----CSEFAGLRQAVT-Q
3vbsC          TMKLCKDASDILQTGTIQ
3vbsD          -----------------K
```

Phylogenetic analysis of VP1



Sc = 8.40
RMS = 0.86

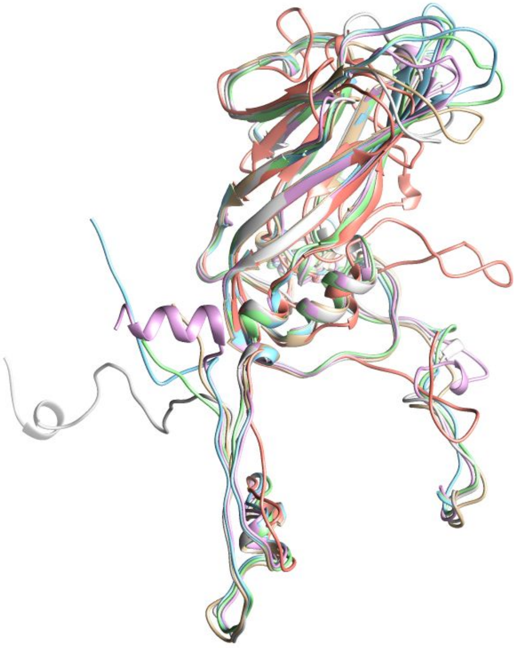
Sc = 8.62
RMS = 0.99

Sc = 7.70
RMS = 1.00

Sc = 7.58
RMS = 0.90

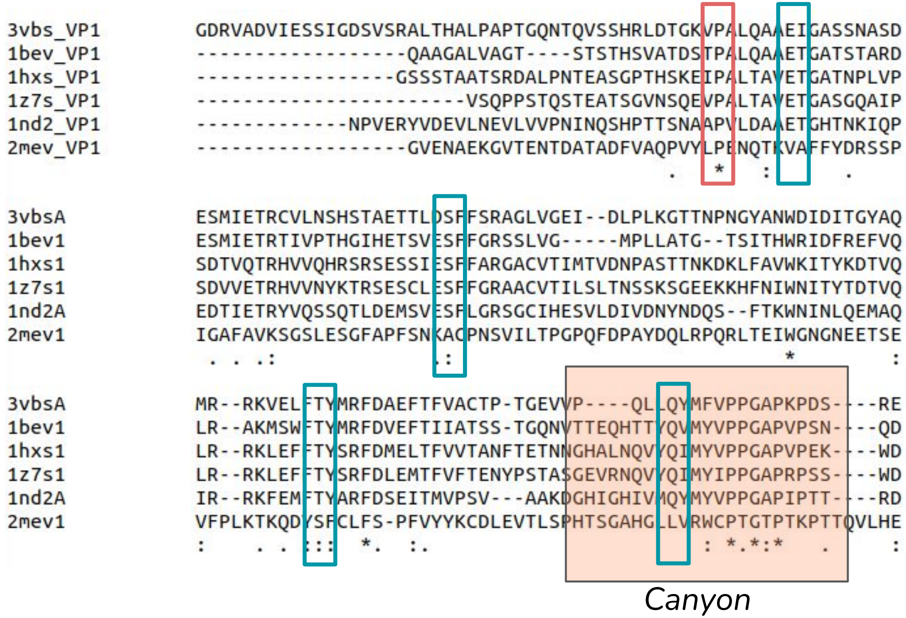
Sc = 5.16
RMS = 1.88

1hxs
1z7s
1nd2
3vbs
1bev
2mev

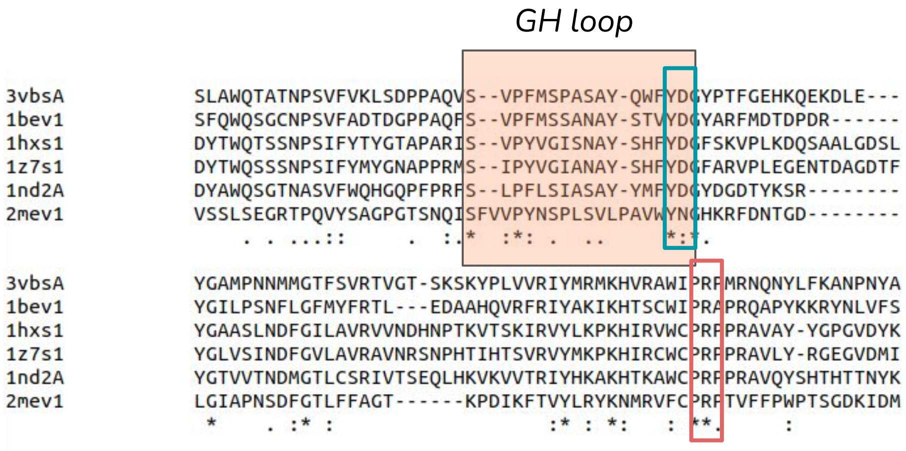


2mev = Mengo Encephalomyelitis Virus
1nd2 = Human Rhinovirus 16
1hxs = Human Poliovirus 1 Mahoney
1z7s = Coxsackievirus A21
3vbs = Human Enterovirus 71
1bev = Bovine Enterovirus strain VG-5-27

MSA of VP1

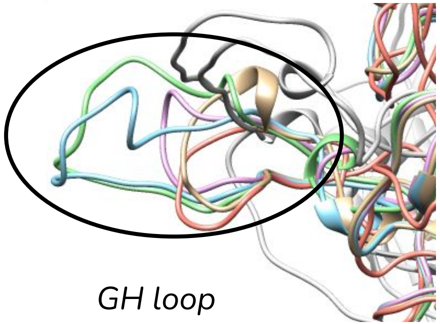


```
3vbs_VP1        GDRVADVIESSIGDSVSRALTHALPAPTGQNTQVSSHRLDTGKVPALQAAEIGASSNASD
1bev_VP1        ------------------QAAGALVAGT----STSTHSVATDSTPALQAAETGATSTARD
1hxs_VP1        -----------------GSSSTAATSRDALPNTEASGPTHSKEIPALTAVETGATNPLVP
1z7s_VP1        -----------------------VSQPPSTQSTEATSGVNSQEVPALTAVETGASGQAIP
1nd2_VP1        -------------NPVERYVDEVLNEVLVVPNINQSHPTTSNAAPVLDAAETGHTNKIQP
2mev_VP1        ------------------GVENAEKGVTENTDATADFVAQPVYLPENQTKVAFFYDRSSP
                                                        .   *   :      .

3vbsA           ESMIETRCVLNSHSTAETTLDSFFSRAGLVGEI--DLPLKGTTNPNGYANWDIDITGYAQ
1bev1           ESMIETRTIVPTHGIHETSVESFFGRSSLVG-----MPLLATG--TSITHWRIDFREFVQ
1hxs1           SDTVQTRHVVQHRSRSESSIESFFARGACVTIMTVDNPASTTNKDKLFAVWKITYKDTVQ
1z7s1           SDVVETRHVVNYKTRSESCLESFFGRAACVTILSLTNSSKSGEEKKHFNIWNITYTDTVQ
1nd2A           EDTIETRYVQSSQTLDEMSVESFLGRSGCIHESVLDIVDNYNDQS--FTKWNINLQEMAQ
2mev1           IGAFAVKSGSLESGFAPFSNKACPNSVILTPGPQFDPAYDQLRPQRLTEIWGNGNEETSE
                 . . .:             .:                            *        :

3vbsA           MR--RKVELFTYMRFDAEFTFVACTP-TGEVVP----QLLQYMFVPPGAPKPDS----RE
1bev1           LR--AKMSWFTYMRFDVEFTIIATSS-TGQNVTTEQHTTYQVMYVPPGAPVPSN----QD
1hxs1           LR--RKLEFFTYSRFDMELTFVVTANFTETNNGHALNQVYQIMYVPPGAPVPEK----WD
1z7s1           LR--RKLEFFTYSRFDLEMTFVFTENYPSTASGEVRNQVYQIMYIPPGAPRPSS----WD
1nd2A           IR--RKFEMFTYARFDSEITMVPSV---AAKDGHIGHIVMQYMYVPPGAPIPTT----RD
2mev1           VFPLKTKQDYSFCLFS-PFVYYKCDLEVTLSPHTSGAHGLLVRWCPTGTPTKPTTQVLHE
                :    . . :::  *.  :.                       : *.*:*   .     :
```

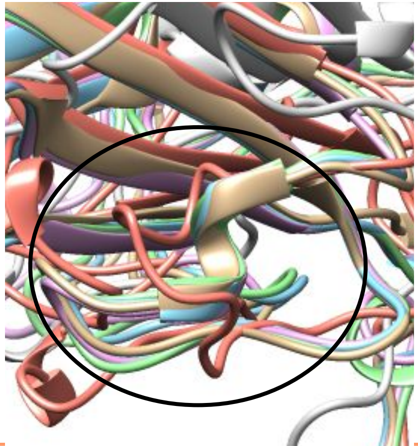


```
3vbsA           SLAWQTATNPSVFVKLSDPPAQVS--VPFMSPASAY-QWFYDGYPTFGEHKQEKDLE---
1bev1           SFQWQSGCNPSVFADTDGPPAQFS--VPFMSSANAY-STVYDGYARFMDTDPDR------
1hxs1           DYTWQTSSNPSIFYTYGTAPARIS--VPYVGISNAY-SHFYDGFSKVPLKDQSAALGDSL
1z7s1           DYTWQSSSNPSIFYMYGNAPPRMS--IPYVGIANAY-SHFYDGFARVPLEGENTDAGDTF
1nd2A           DYAWQSGTNASVFWQHGQPFPRFS--LPFLSIASAY-YMFYDGYDGDTYKSR--------
2mev1           VSSLSEGRTPQVYSAGPGTSNQISFVVPYNSPLSVLPAVWYNGHKRFDNTGD--------
                    . . ...::     .  :.*  :*: .  ..     *:*.

3vbsA           YGAMPNNMMGTFSVRTVGT-SKSKYPLVVRIYMRMKHVRAWIPRFMRNQNYLFKANPNYA
1bev1           YGILPSNFLGFMYFRTL---EDAAHQVRFRIYAKIKHTSCWIPRAPRQAPYKKRYNLVFS
1hxs1           YGAASLNDFGILAVRVVNDHNPTKVTSKIRVYLKPKHIRVWCPRPPRAVAY-YGPGVDYK
1z7s1           YGLVSINDFGVLAVRAVNRSNPHTIHTSVRVYMKPKHIRCWCPRPPRAVLY-RGEGVDMI
1nd2A           YGTVVTNDMGTLCSRIVTSEQLHKVKVVTRIYHKAKHTKAWCPRPPRAVQYSHTHTTNYK
2mev1           LGIAPNSDFGTLFFAGT------KPDIKFTVYLRYKNMRVFCPRPTVFFPWPTSGDKIDM
                 *    . :* :                  :* : *:   : **.     :

3vbsA           GNSIKP---TGASRTAITTL
1bev1           GDSDR----ICSNRASLTSY
1hxs1           DGTLTP----LSTKD-LTTY
1z7s1           SSAILP----LTKVDSITTF
1nd2A           LSSEVHNDVAIRPRTNLTTV
2mev1           T-------------------
```

Residues conserved among enterovirus (*genus*)
Residues conserved among picornavirus (enterovirus & cardiovirus)

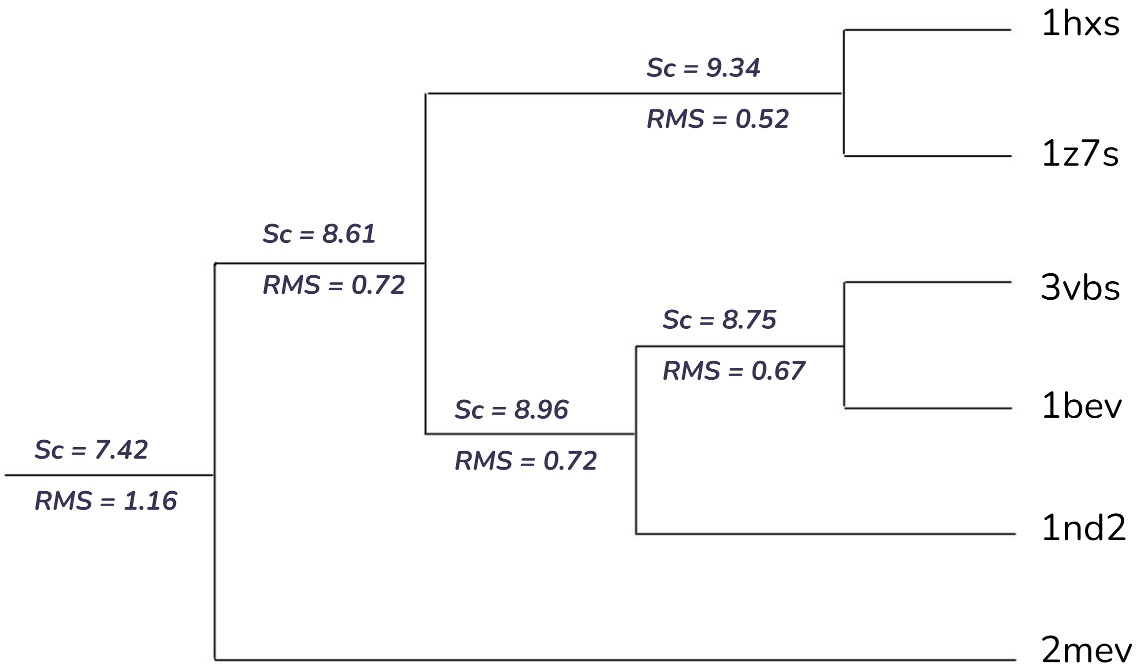


GH loop



Canyon

Phylogenetic analysis of VP2



Sc = 9.34

RMS = 0.52

Sc = 8.61

RMS = 0.72

Sc = 8.75

RMS = 0.67

Sc = 8.96

RMS = 0.72

Sc = 7.42

RMS = 1.16

1hxs

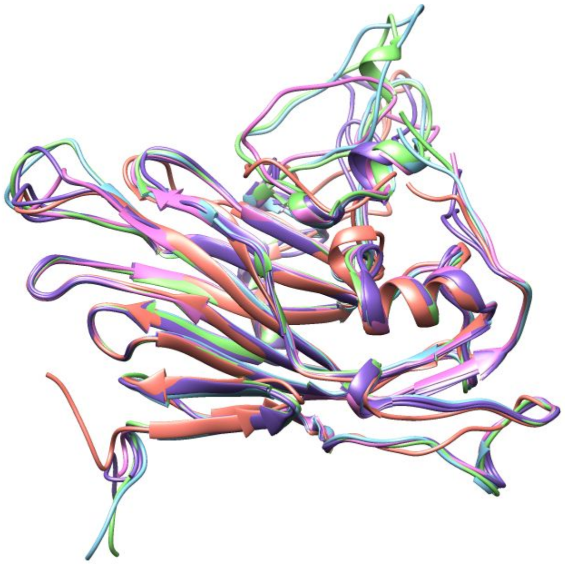
1z7s

3vbs

1bev

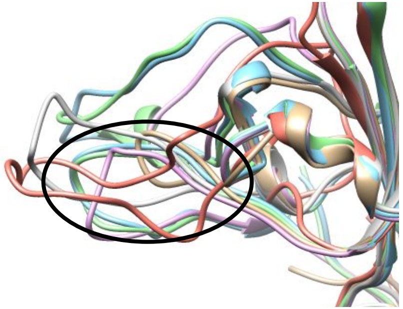
1nd2

2mev

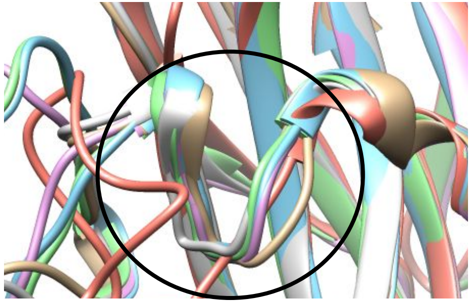


2mev = Mengo Encephalomyelitis Virus
1nd2 = Human Rhinovirus 16
1hxs = Human Poliovirus 1 Mahoney
1z7s = Coxsackievirus A21
3vbs = Human Enterovirus 71
1bev = Bovine Enterovirus strain VG-5-27

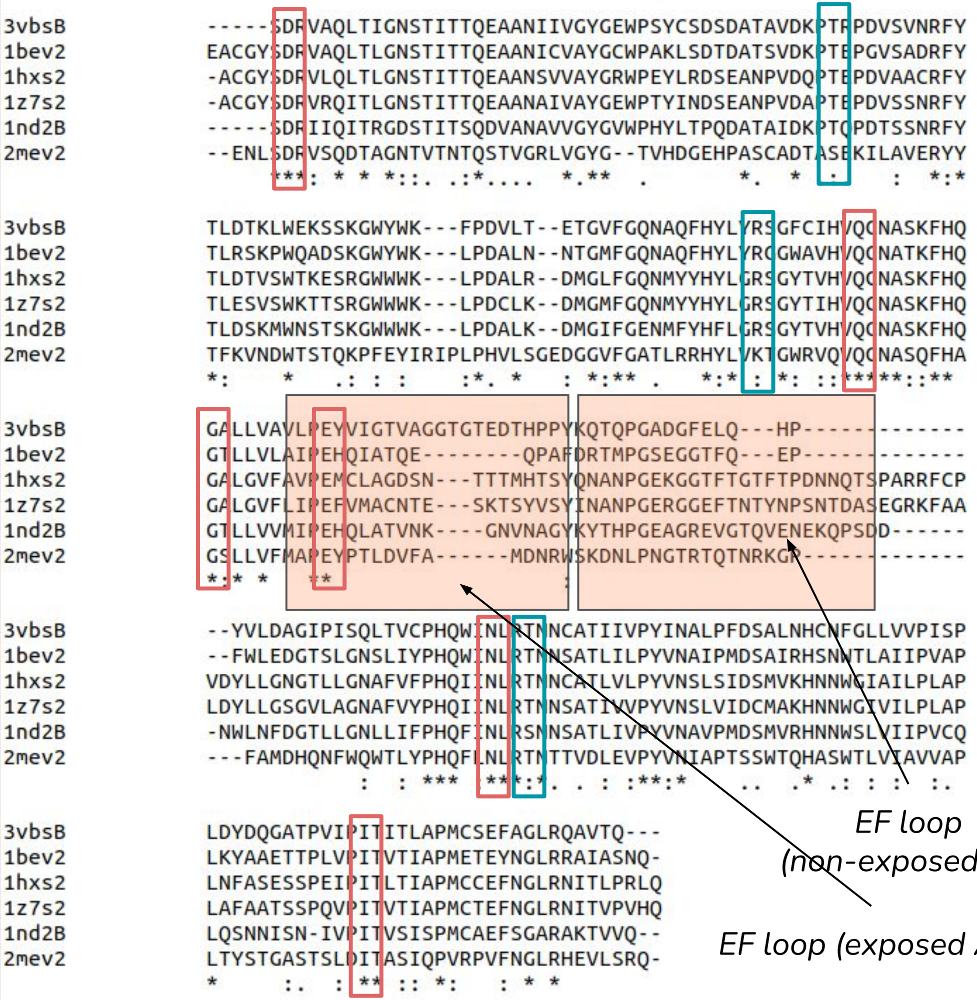
MSA of VP2



Aa exposed of EF loop



Aa not exposed of EF loop



```
3vbsB           -----SDRVAQLTIGNSTITTQEAANIIVGYGEWPSYCSDSDATAVDKPTRPDVSVNRFY
1bev2           EACGYSDRVAQLTLGNSTITTQEAANICVAYGCWPAKLSDTDATSVDKPTEPGVSADRFY
1hxs2           -ACGYSDRVLQLTLGNSTITTQEAANSVVAYGRWPEYLRDSEANPVDQPTEPDVAACRFY
1z7s2           -ACGYSDRVRQITLGNSTITTQEAANAIVAYGEWPTYINDSEANPVDAPTEPDVSSNRFY
1nd2B           -----SDRIIQITRGDSTITSQDVANAVVGYGVWPHYLTPQDATAIDKPTQPDTSSNRFY
2mev2           --ENLSDRVSQDTAGNTVTNTQSTVGRLVGYG--TVHDGEHPASCADTASEKILAVERYY
                     ***: * * *::. .:*....  *.**  .       *.  *  .    :  *:*

3vbsB           TLDTKLWEKSSKGWYWK---FPDVLT--ETGVFGQNAQFHYLYRSGFCIHVQQNASKFHQ
1bev2           TLRSKPWQADSKGWYWK---LPDALN--NTGMFGQNAQFHYLYRGGWAVHVQQNATKFHQ
1hxs2           TLDTVSWTKESRGWWWK---LPDALR--DMGLFGQNMYYHYLGRSGYTVHVQQNASKFHQ
1z7s2           TLESVSWKTTSRGWWWK---LPDCLK--DMGMFGQNMYYHYLGRSGYTIHVQQNASKFHQ
1nd2B           TLDSKMWNSTSKGWWWK---LPDALK--DMGIFGENMFYHFLGRSGYTVHVQQNASKFHQ
2mev2           TFKVNDWTSTQKPFEYIRIPLPHVLSGEDGGVFGATLRRHYLVKTGWRVQVQQNASQFHA
                *:    *   .: : :    :*. *   : *:** .   *:* : *: ::*****::**

3vbsB           GALLVAVLPEYVIGTVAGGTGTEDTHPPYKQTQPGADGFELQ---HP-------------
1bev2           GTLLVLAIPEHQIATQE--------QPAFDRTMPGSEGGTFQ---EP-------------
1hxs2           GALGVFAVPEMCLAGDSN---TTTMHTSYQNANPGEKGGTFTGTFTPDNNQTSPARRFCP
1z7s2           GALGVFLIPEFVMACNTE---SKTSYVSYINANPGERGGEFTNTYNPSNTDASEGRKFAA
1nd2B           GTLLVVMIPEHQLATVNK----GNVNAGYKYTHPGEAGREVGTQVENEKQPSDD------
2mev2           GSLLVFMAPEYPTLDVFA------MDNRWSKDNLPNGTRTQTNRKGP-------------
                *:* *   **                  :

3vbsB           --YVLDAGIPISQLTVCPHQWINLRTNNCATIIVPYINALPFDSALNHCNFGLLVVPISP
1bev2           --FWLEDGTSLGNSLIYPHQWINLRTNNSATLILPYVNAIPMDSAIRHSNWTLAIIPVAP
1hxs2           VDYLLGNGTLLGNAFVFPHQIINLRTNNCATLVLPYVNSLSIDSMVKHNNWGIAILPLAP
1z7s2           LDYLLGSGVLAGNAFVYPHQIINLRTNNSATIVVPYVNSLVIDCMAKHNNWGIVILPLAP
1nd2B           -NWLNFDGTLLGNLLIFPHQFINLRSNNSATLIVPYVNAVPMDSMVRHNNWSLVIIPVCQ
2mev2           ---FAMDHQNFWQWTLYPHQFLNLRTNTTVDLEVPYVNIAPTSSWTQHASWTLVIAVVAP
            :  : ***  :***:*. . : :**:*    ..  .* .: : :  :.

3vbsB           LDYDQGATPVIPITITLAPMCSEFAGLRQAVTQ---
1bev2           LKYAAETTPLVPITVTIAPMETEYNGLRRAIASNQ-
1hxs2           LNFASESSPEIPITLTIAPMCCEFNGLRNITLPRLQ
1z7s2           LAFAATSSPQVPITVTIAPMCTEFNGLRNITVPVHQ
1nd2B           LQSNNISN-IVPITVSISPMCAEFSGARAKTVVQ--
2mev2           LTYSTGASTSLDITASIQPVRPVFNGLRHEVLSRQ-
                *     :.  : ** :: *:   : * *
```

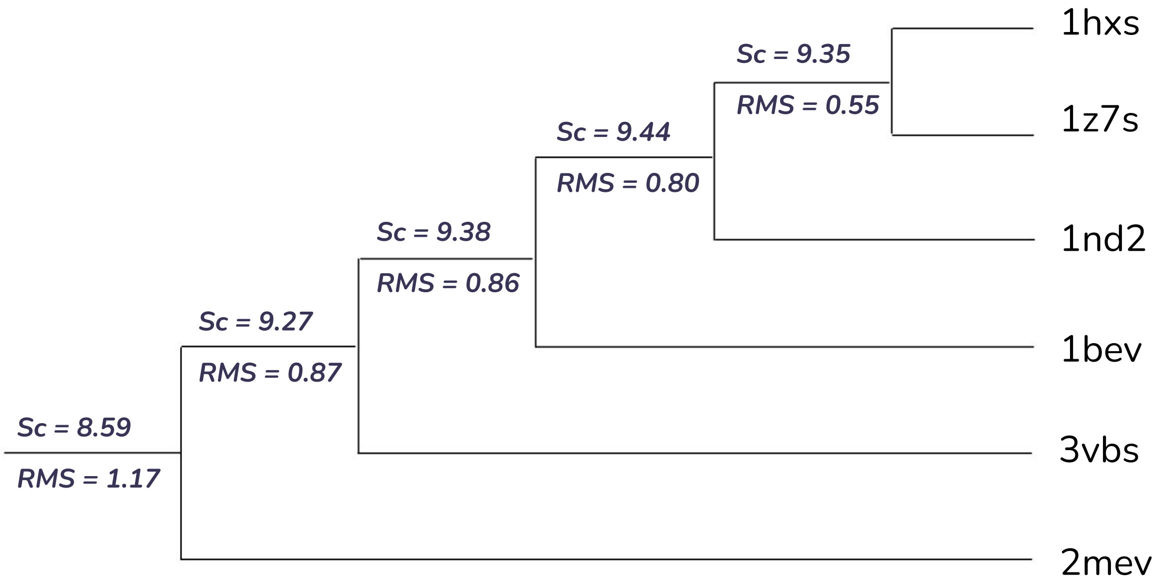
EF loop (non-exposed Aa)

EF loop (exposed Aa)

Residues conserved among enterovirus (genus)

Residues conserved among picornavirus (enterovirus & cardiovirus)

Phylogenetic analysis of VP3



Sc = 9.35

RMS = 0.55

Sc = 9.44

RMS = 0.80

Sc = 9.38

RMS = 0.86

Sc = 9.27

RMS = 0.87

Sc = 8.59

RMS = 1.17

1hxs

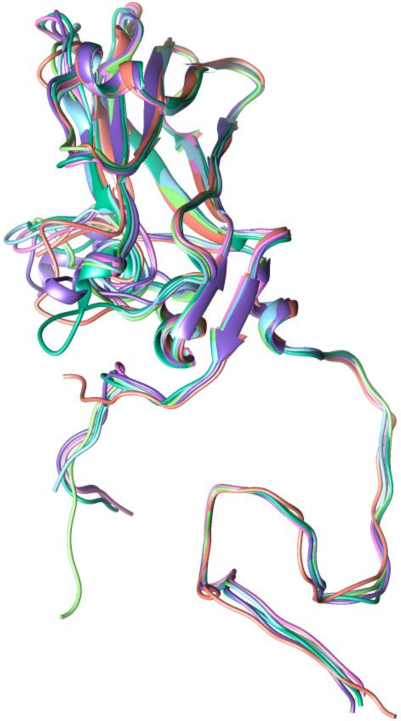
1z7s

1nd2

1bev

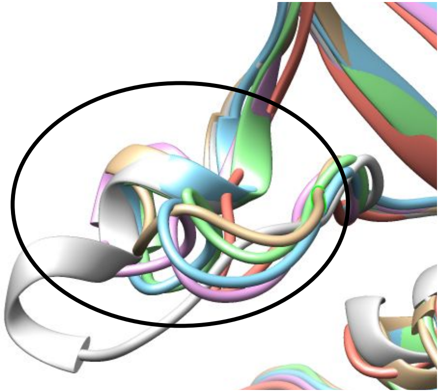
3vbs

2mev

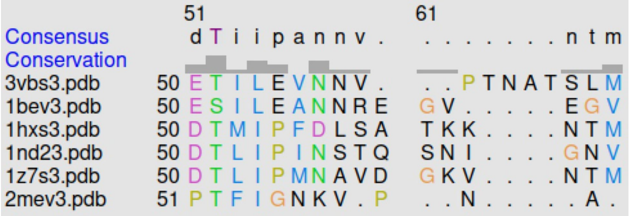


2mev = Mengo Encephalomyelitis Virus
1nd2 = Human Rhinovirus 16
1hxs = Human Poliovirus 1 Mahoney
1z7s = Coxsackievirus A21
3vbs = Human Enterovirus 71
1bev = Bovine Enterovirus strain VG-5-27

MSA of VP3



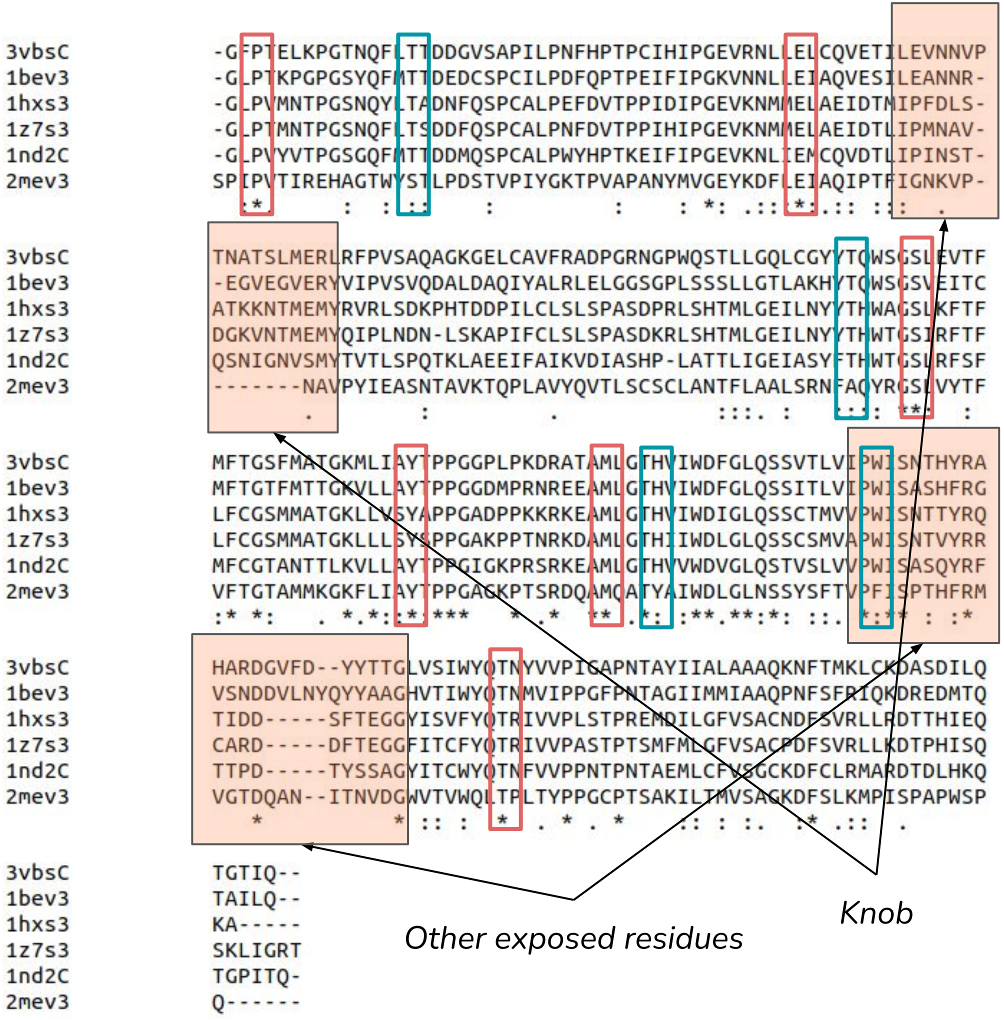
Knob



```
                  51                  61
Consensus         d T i i p a n n v .  . . . . . . . n t m
Conservation
3vbs3.pdb    50   E T I L E V N N V .  . . P T N A T S L M
1bev3.pdb    50   E S I L E A N N R E  G V . . . . . E G V
1hxs3.pdb    50   D T M I P F D L S A  T K K . . . . . N T M
1nd23.pdb    50   D T L I P I N S T Q  S N I . . . . . G N V
1z7s3.pdb    50   D T L I P M N A V D  G K V . . . . . N T M
2mev3.pdb    51   P T F I G N K V . P  . . N . . . . . . A .
```

Residues conserved among enterovirus (genus)

Residues conserved among picornavirus (enterovirus & cardiovirus)

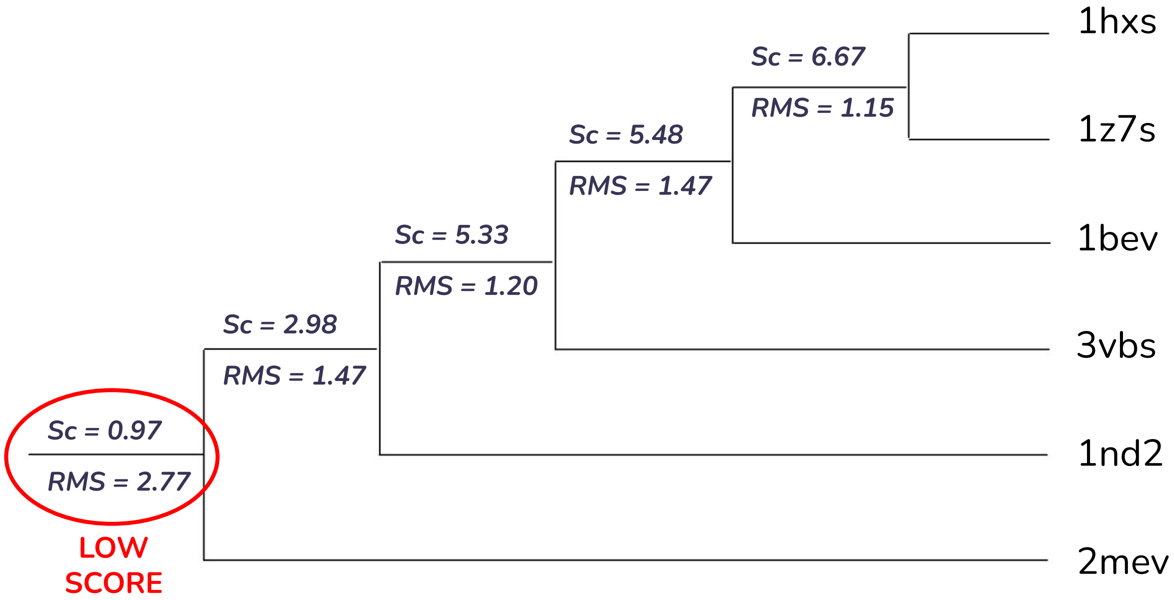


```
3vbsC      -GFPTELKPGTNQFLTTDDGVSAPILPNFHPTPCIHIPGEVRNLLELCQVETILEVNNVP
1bev3      -GLPTKPGPGSYQFMTTDEDCSPCILPDFQPTPEIFIPGKVNNLLEIAQVESILEANNR-
1hxs3      -GLPVMNTPGSNQYLTADNFQSPCALPEFDVTPPIDIPGEVKNMMELAEIDTMIPFDLS-
1z7s3      -GLPTMNTPGSNQFLTSDDFQSPCALPNFDVTPPIHIPGEVKNMMELAEIDTLIPMNAV-
1nd2C      -GLPVYVTPGSGQFMTTDDMQSPCALPWYHPTKEIFIPGEVKNLIEMCQVDTLIPINST-
2mev3      SPIPVTIREHAGTWYSTLPDSTVPIYGKTPVAPANYMVGEYKDFLEIAQIPTFIGNKVP-
             :*.     :  : ::    :         :    : *: .::::*:.:: :::  .

3vbsC      TNATSLMERLRFPVSAQAGKGELCAVFRADPGRNGPWQSTLLGQLCGYYTQWSGSLEVTF
1bev3      -EGVEGVERYVIPVSVQDALDAQIYALRLELGGSGPLSSSLLGTLAKHYTQWSGSVEITC
1hxs3      ATKKNTMEMYRVRLSDKPHTDDPILCLSLSPASDPRLSHTMLGEILNYYTHWAGSLKFTF
1z7s3      DGKVNTMEMYQIPLNDN-LSKAPIFCLSLSPASDKRLSHTMLGEILNYYTHWTGSIRFTF
1nd2C      QSNIGNVSMYTVTLSPQTKLAEEIFAIKVDIASHP-LATTLIGEIASYFTHWTGSLRFSF
2mev3      -------NAVPYIEASNTAVKTQPLAVYQVTLSCSCLANTFLAALSRNFAQYRGSLVYTF
                  .        :         .            :::. :   :::: **:  :

3vbsC      MFTGSFMATGKMLIAYTPPGGPLPKDRATAMLGTHVIWDFGLQSSVTLVIPWISNTHYRA
1bev3      MFTGTFMTTGKVLLAYTPPGGDMPRNREEAMLGTHVIWDFGLQSSITLVIPWISASHFRG
1hxs3      LFCGSMMATGKLLVSYAPPGADPPKKRKEAMLGTHVIWDIGLQSSCTMVVPWISNTTYRQ
1z7s3      LFCGSMMATGKLLLSYSPPGAKPPTNRKDAMLGTHIIWDLGLQSSCSMVAPWISNTVYRR
1nd2C      MFCGTANTTLKVLLAYTPPGIGKPRSRKEAMLGTHVVWDVGLQSTVSLVVPWISASQYRF
2mev3      VFTGTAMMKGKFLIAYTPPGAGKPTSRDQAMQATYAIWDLGLNSSYSFTVPFISPTHFRM
           :* *:   . *.*::*:***   * .*  ** .*: :**.**:*: ::. *:** : :*

3vbsC      HARDGVFD--YYTTGLVSIWYQTNYVVPIGAPNTAYIIALAAAQKNFTMKLCKDASDILQ
1bev3      VSNDDVLNYQYYAAGHVTIWYQTNMVIPPGFPNTAGIIMMIAAQPNFSFRIQKDREDMTQ
1hxs3      TIDD-----SFTEGGYISVFYQTRIVVPLSTPREMDILGFVSACNDFSVRLLRDTTHIEQ
1z7s3      CARD-----DFTEGGFITCFYQTRIVVPASTPTSMFMLGFVSACPDFSVRLLKDTPHISQ
1nd2C      TTPD-----TYSSAGYITCWYQTNFVVPPNTPNTAEMLCFVSGCKDFCLRMARDTDLHKQ
2mev3      VGTDQAN--ITNVDGWVTVWQLTPLTYPPGCPTSAKILTMVSAGKDFSLKMPISPAPWSP
              *          * :: :  *  . * . *    :: : :.  :* .::  .

3vbsC      TGTIQ--
1bev3      TAILQ--
1hxs3      KA-----
1z7s3      SKLIGRT
1nd2C      TGPITQ-
2mev3      Q------
```

Other exposed residues

Knob

Phylogenetic analysis of VP4



Sc = 0.97

RMS = 2.77

LOW SCORE

Sc = 2.98

RMS = 1.47

Sc = 5.33

RMS = 1.20

Sc = 5.48

RMS = 1.47

Sc = 6.67

RMS = 1.15

1hxs

1z7s

1bev

3vbs

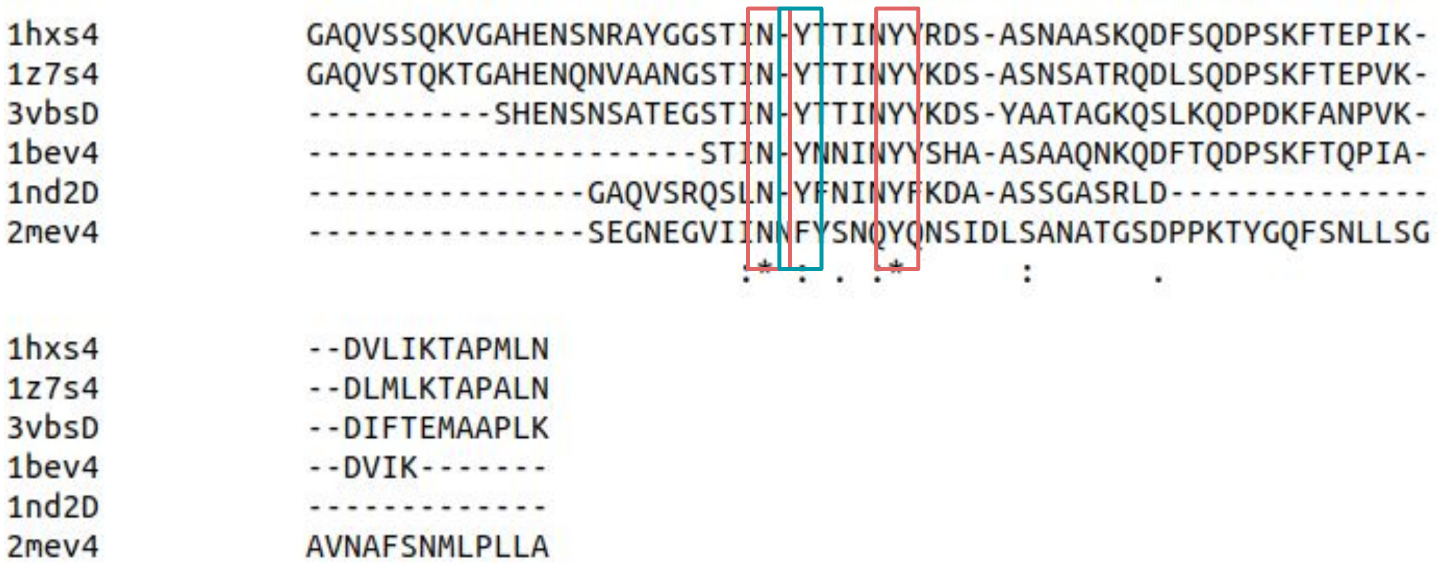
1nd2

2mev

2mev = Mengo Encephalomyelitis Virus
1nd2 = Human Rhinovirus 16
1hxs = Human Poliovirus 1 Mahoney
1z7s = Coxsackievirus A21
3vbs = Human Enterovirus 71
1bev = Bovine Enterovirus strain VG-5-27

MSA of VP4

Residues conserved among enterovirus (genus)
Residues conserved among picornavirus (enterovirus & cardiovirus)

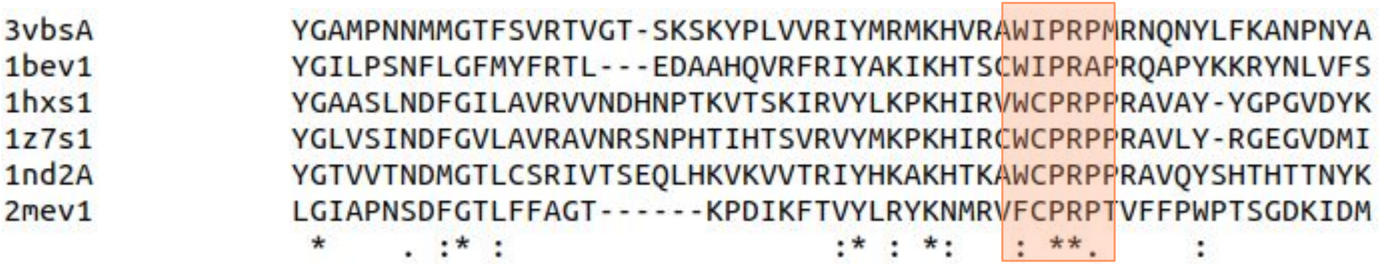


```
1hxs4           GAQVSSQKVGAHENSNRAYGGSTIN-YTTINYYRDS-ASNAASKQDFSQDPSKFTEPIK-
1z7s4           GAQVSTQKTGAHENQNVAANGSTIN-YTTINYYKDS-ASNSATRQDLSQDPSKFTEPVK-
3vbsD           ----------SHENSNSATEGSTIN-YTTINYYKDS-YAATAGKQSLKQDPDKFANPVK-
1bev4           ---------------------STIN-YNNINYYSHA-ASAAQNKQDFTQDPSKFTQPIA-
1nd2D           ---------------GAQVSRQSLN-YFNINYFKDA-ASSGASRLD--------------
2mev4           ---------------SEGNEGVIINNFYSNQYQNSIDLSANATGSDPPKTYGQFSNLLSG
                                       :* : . .*      :      .

1hxs4           --DVLIKTAPMLN
1z7s4           --DLMLKTAPALN
3vbsD           --DIFTEMAAPLK
1bev4           --DVIK-------
1nd2D           -------------
2mev4           AVNAFSNMLPLLA
```

Search of relevant functional motifs: WCPRP example

There is a critical region, highly conserved among picornaviruses, involved in the correct processing of the capsid precursor: WCPRP in enteroviruses and FCPRP in cardioviruses.

ClustalW → extreme conservation of the motif



```
3vbsA           YGAMPNNMMGTFSVRTVGT-SKSKYPLVVRIYMRMKHVRAWIPRPMRNQNYLFKANPNYA
1bev1           YGILPSNFLGFMYFRTL---EDAAHQVRFRIYAKIKHTSCWIPRAPRQAPYKKRYNLVFS
1hxs1           YGAASLNDFGILAVRVVNDHNPTKVTSKIRVYLKPKHIRVWCPRPPRAVAY-YGPGVDYK
1z7s1           YGLVSINDFGVLAVRAVNRSNPHTIHTSVRVYMKPKHIRCWCPRPPRAVLY-RGEGVDMI
1nd2A           YGTVVTNDMGTLCSRIVTSEQLHKVKVVTRIYHKAKHTKAWCPRPPRAVQYSHTHTTNYK
2mev1           LGIAPNSDFGTLFFAGT------KPDIKFTVYLRYKNMRVFCPRPTVFFPWPTSGDKIDM
                 *    . :* :                  :* : *:   : **.     :
```

Some key conclusions...

As for structure and interactions:

- There are many intraprotomeric and interprotomeric interactions, in form of H bond, contributing to many key processes of the virus: capsid assembly, infectivity...
- We were also able to find some salt bridges, but no disulfide bonds
- VP1's GH loop, VP3's knob and other relevant parts of the canyon are very conserved, very likely due to their relevance for the virus
- The ALD inhibitor has a great therapeutic potential due to its sphingosine-specific mechanism of action

Some key conclusions...

As for phylogeny:

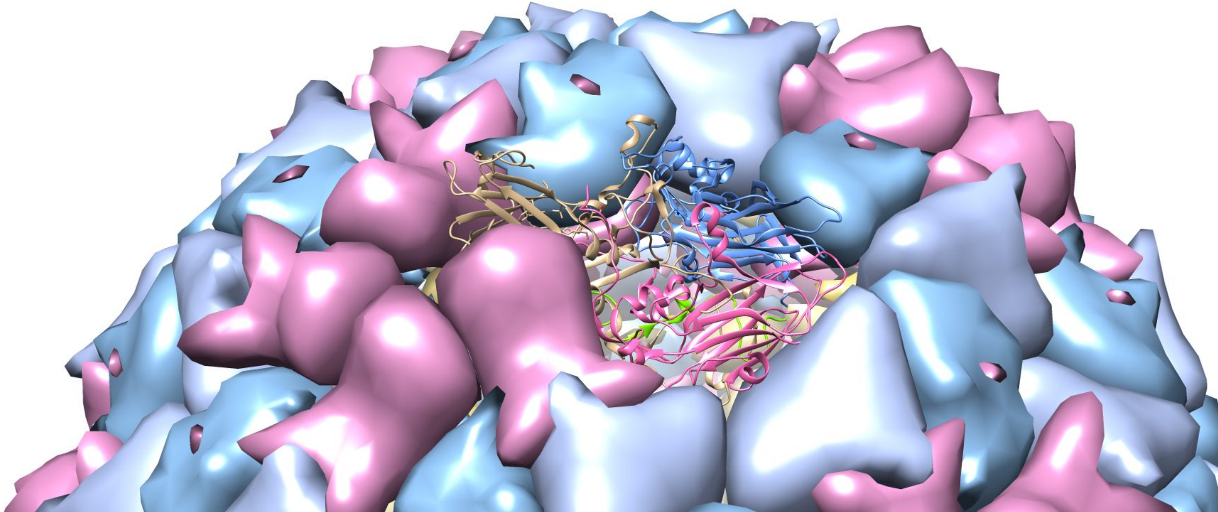
- VP1, VP2 and VP3 are paralogous due to duplication events prior to the divergence of Picornavirales
- In our virus, VP3 is the most conserved and VP4 the least, according to structural and sequence similarity with our templates
- In general, some regions of these VPs are more conserved than others (i.e. the middle region is more conserved than the extremes, which is where the loops take place)
- Sc and RMSD values show high structural similarity and evolutionary relationship → many of the most conserved residues correspond to residues involved in H bonds or salt bridges, also structurally relevant regions such as the canyon

Some key conclusions...

As an extra remark...

More research on the structural characteristics of Human Enterovirus 71 and its related species should be pursued to overcome the lack of information available on the functions of many regions and bonds

THANK YOU FOR YOUR ATTENTION!



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PEM questions

1. The Human Enterovirus 71 (HEV71) belongs to the family of:

   a. Picornaviridae
   b. Retroviridae
   c. Herpesviridae
   d. Rhabdoviridae
   e. Adenoviridae

2. Choose the correct statement about HEV71:

   a. Its capsid protomer is composed by 3 viral proteins (VP1, VP2 and VP3)
   b. Almost all of the viral proteins have a jelly roll supersecondary structure
   c. Options a) and b) are true
   d. It has a canyon
   e. All of the above are true

3. The most dissimilar viral protein in structure is:

   a. VP1
   b. VP2
   c. VP3
   d. VP4
   e. All of them are very dissimilar in structure

PEM questions

4. Which of the following is true:

a. The triangulation number gives information about the size of the capsid
b. The higher the triangulation number, the more protomers compose the capsid
c. Options a) and b) are correct
d. All icosahedral viruses have the same number of capsid proteins
e. All of the above are correct

5. Which of the following is false:

a. Inter-protomeric interactions occur between two different protomers (capsid units)
b. There are several interactions found between different protomers, mainly hydrogen bonds
c. Intra-protomeric interactions occur between proteins within the same protomer (capsid unit)
d. There are no interactions between neighbouring capsomers, interactions only occur among proteins of the same capsomer
e. None of the previous options are false (all of them are true statements)

6. SCOP classifies HEV71 proteins as:

a. All-beta proteins
b. All-alpha proteins
c. Alpha+beta proteins
d. Alpha/beta proteins
e. Small proteins

PEM questions

7. HEV71 capsid protomer:

a. It is composed by 3 viral proteins
b. It rearranges to make a pore for genome release
c. Forms pentamers
d. When in pentamer conformation, 12 of them associate to form the procapsid
e. [✓ All of the above are true]

8. VP1 has:

a. The knob
b. The puff
c. [✓ A GH loop very relevant for receptor binding]
d. None of the above
e. All of the above

9. About symmetry and axis:

a. HEV71 has only a 2-fold axis
b. HEV71 has only a 3-fold axis
c. HEV71 has only a 5-fold axis
d. [✓ HEV71 has a 2-fold, a 3-fold and a 5-fold axis]
e. HEV71 has a 7-fold axis

PEM questions

10. About the ALD inhibitor, for HEV71:

a. It replaces the sphingosine of the hydrophobic pocket
b. It’s main key interaction is established between the amide group and the D112 residue
c. a and b are correct
d. It contains a phenoxy group which helps the binding with the pocket
e. All are correct