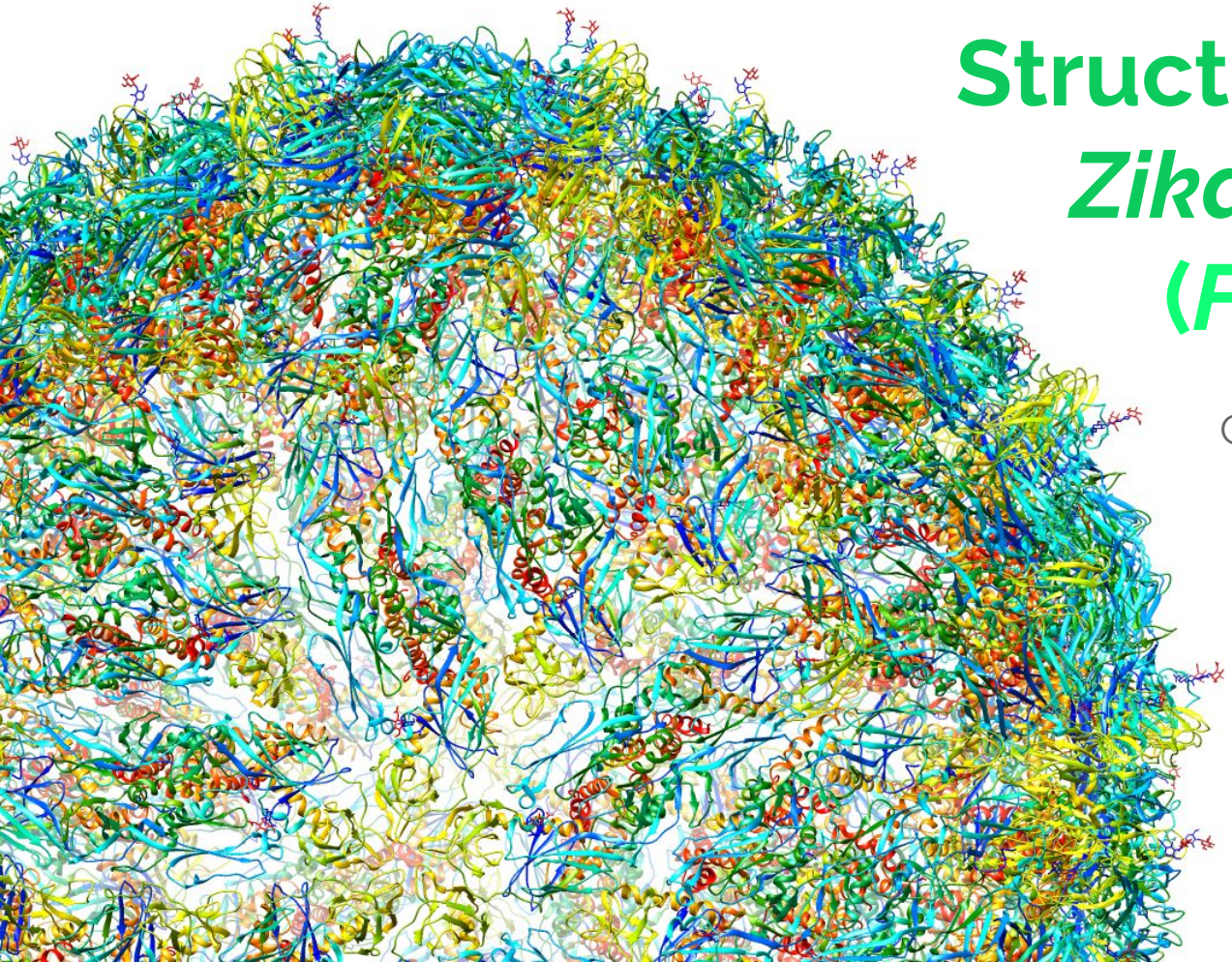
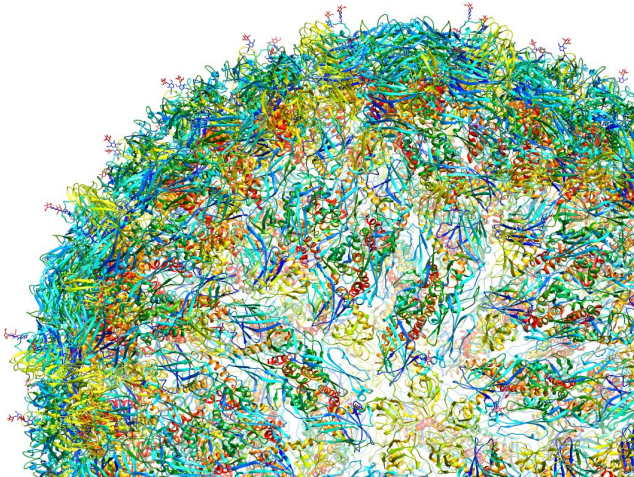


Structure of *Zika virus* (*Flavivirus*)

Campos M, Canales A,
Díaz J, Llerena M.

Structural Biology
Human Biology, UPF
2018-2019

Index



FLAVIVIRIDAE FAMILY

→ Includes a huge number of virus

→ **Genuses**: ***Flavivirus***, *Pestivirus*, *Hepacivirus*

Mosquito-borne viruses

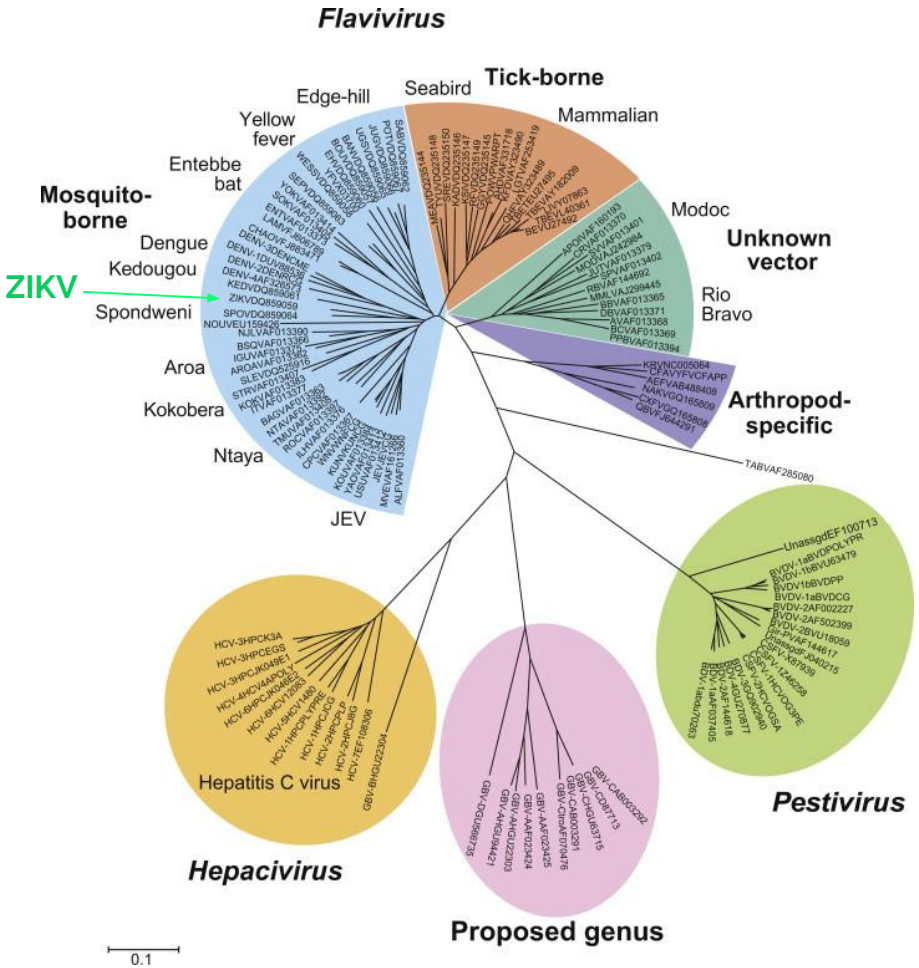
***Zika virus* (ZIKV)**

Dengue virus (DENV)

West Nile virus (WNV)

Japanese encephalitis virus (JEV)

...



Flaviviridae phylogeny.
Source: International Committee on Taxonomy of Viruses (2012)

Zika virus (ZIKV)

PUBLIC HEALTH CONCERN

FEBRUARY 2, 2016

Zika Virus: Global Public Health Emergency

Catalunya afronta un risc moderat-alt de Zika, dengue i Chikungunya, segons l'Organització Mundial de la Salut

ALERTA SANITARIA

Primera muerte de un bebé en EEUU por zika

09/08/2016 - 20:04 CEST

El número de diagnosticados por zika en España se eleva ya a 247

29/08/2016 - 16:09 CEST

Entre los afectados hay 39 embarazadas, una más que en el anterior recuento

Zika: one in seven babies with mothers exposed to virus developed health issues

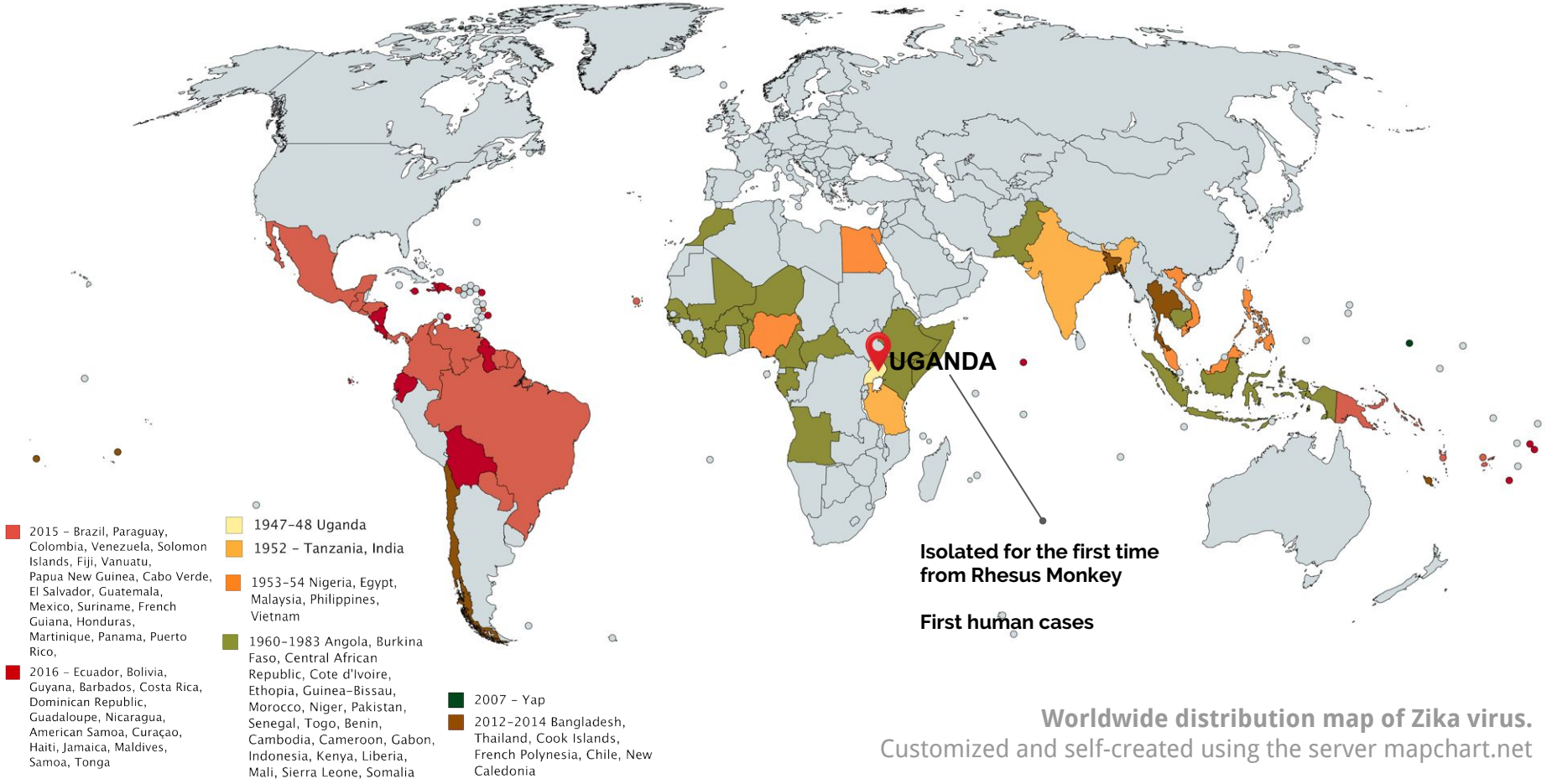
New study is first to examine health of children beyond birth after mothers were exposed to mosquito-borne disease

Detecten quatre nous casos de Zika a Catalunya, entre els quals una embarassada

Amb aquests, el nombre d'infectats a casa nostra s'eleva fins a 45, 19 homes i 26 dones

Acn | Barcelona | 17.05.2016 | 12:33

WORLDWIDE DISTRIBUTION OF *ZIKA VIRUS*



Worldwide distribution map of Zika virus.
Customized and self-created using the server mapchart.net

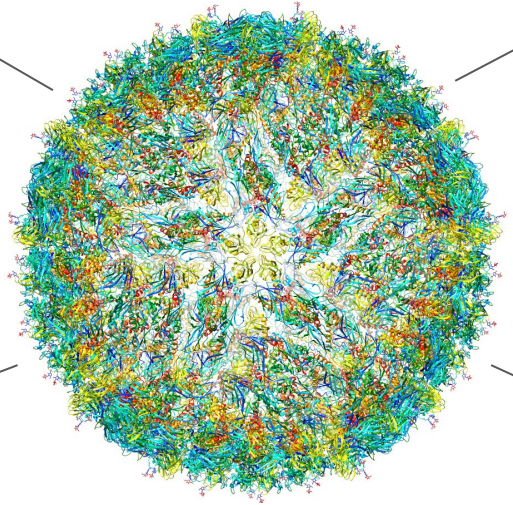
MAIN FEATURES OF *ZIKA VIRUS*

Characteristics:
Flavivirus genus
With icosahedral envelope
Positive stranded RNA virus

Symptoms:
Mild disease, lasting around 2-7 days

(rash, headache, diarrhea, fever, joint and muscle pain, conjuntivitis)

- Low death risk
- Increased **risk of microcephaly** in fetus



NO treatment or vaccine is available

Transmission:

Mosquito bite
(*Aedes aegypti, Aedes albopictus*)
Sexual
Intrauterine
Blood transfusion

Hosts:

Rodents
Pigs
Birds
Primates
Humans

ZIKV STRUCTURE

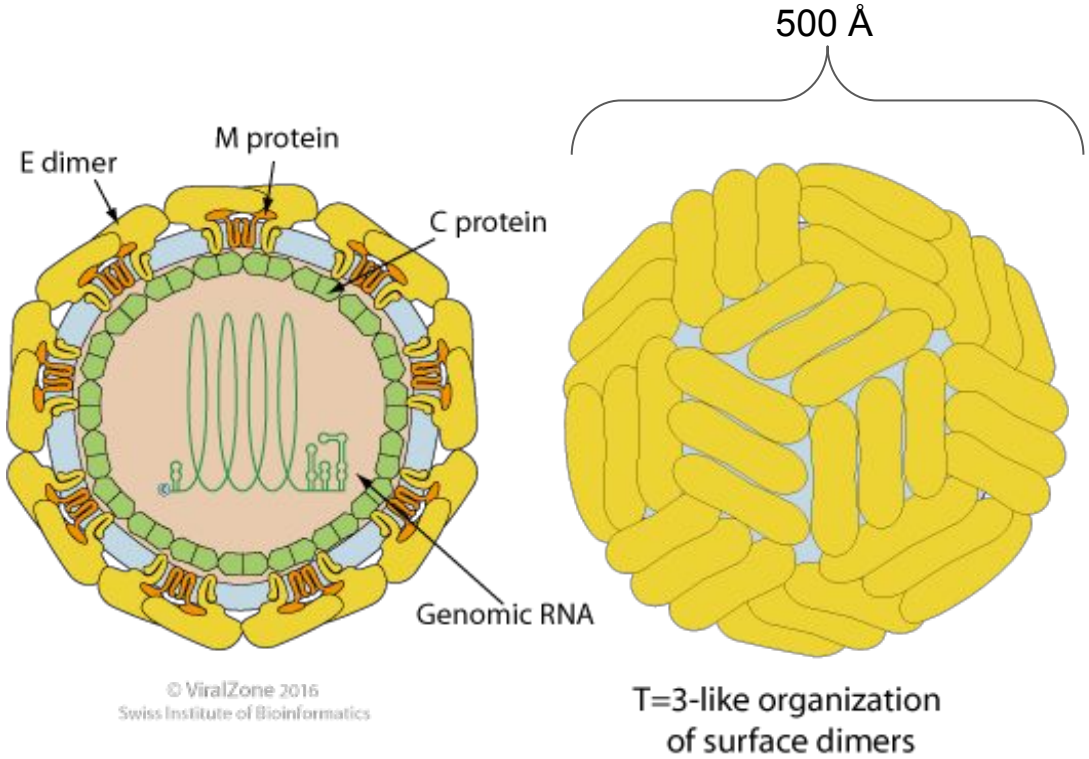
Capsid protein **(C)**

Envelope protein **(E)**

Membrane protein **(M)**

Immature:

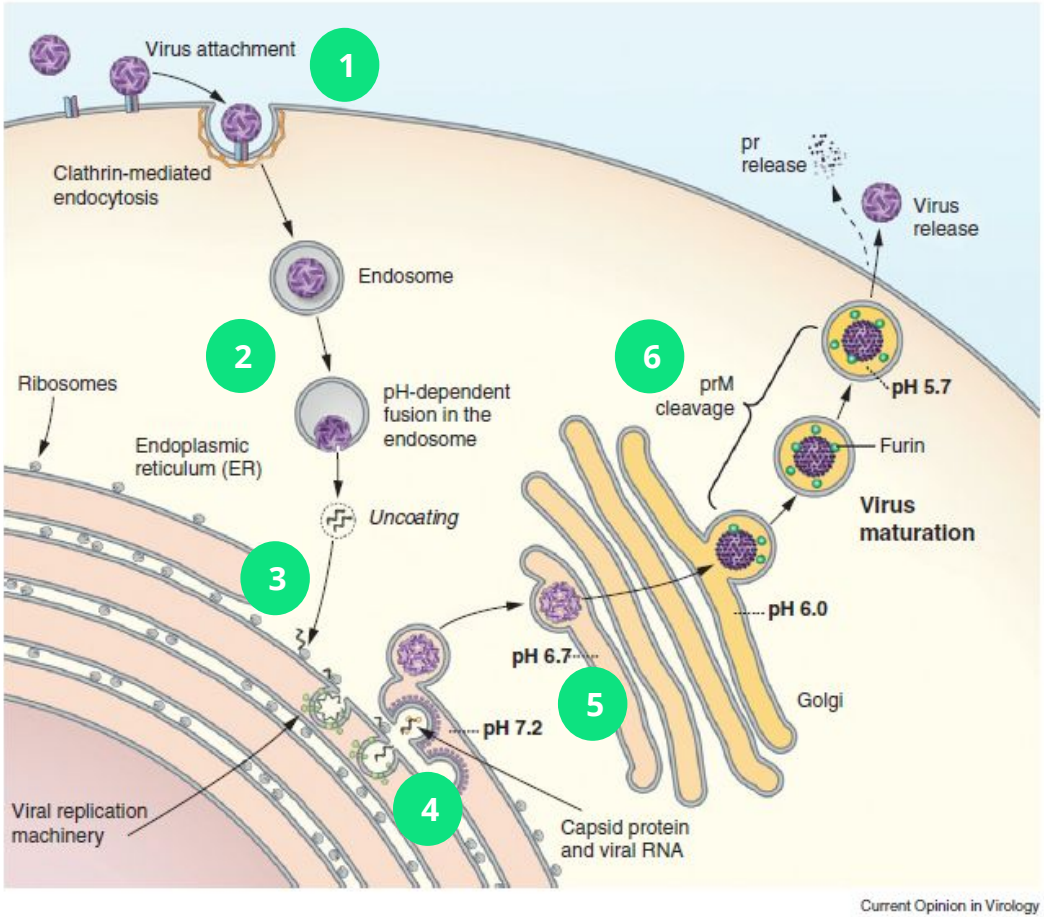
Premembrane protein **(prM)**



Mature flavivirus structure. Modified from ViralZone - Swiss Institute of Bioinformatics (2016)

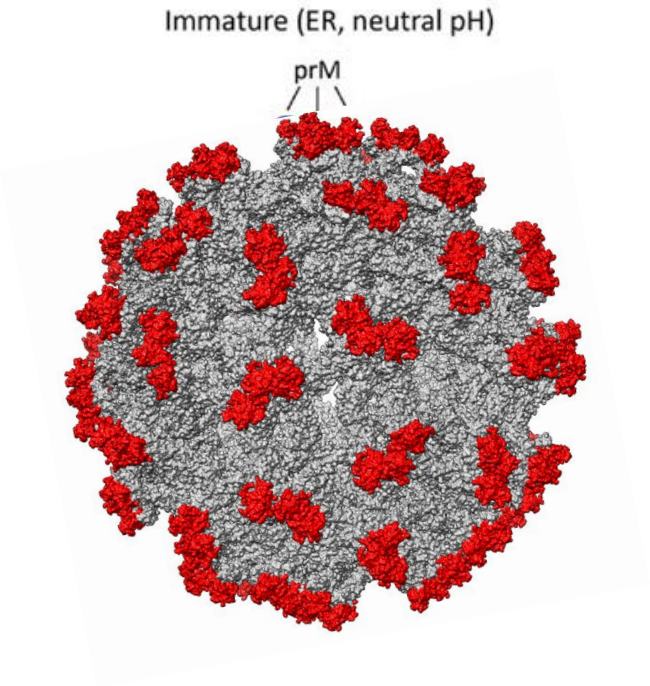
INFECTION CYCLE

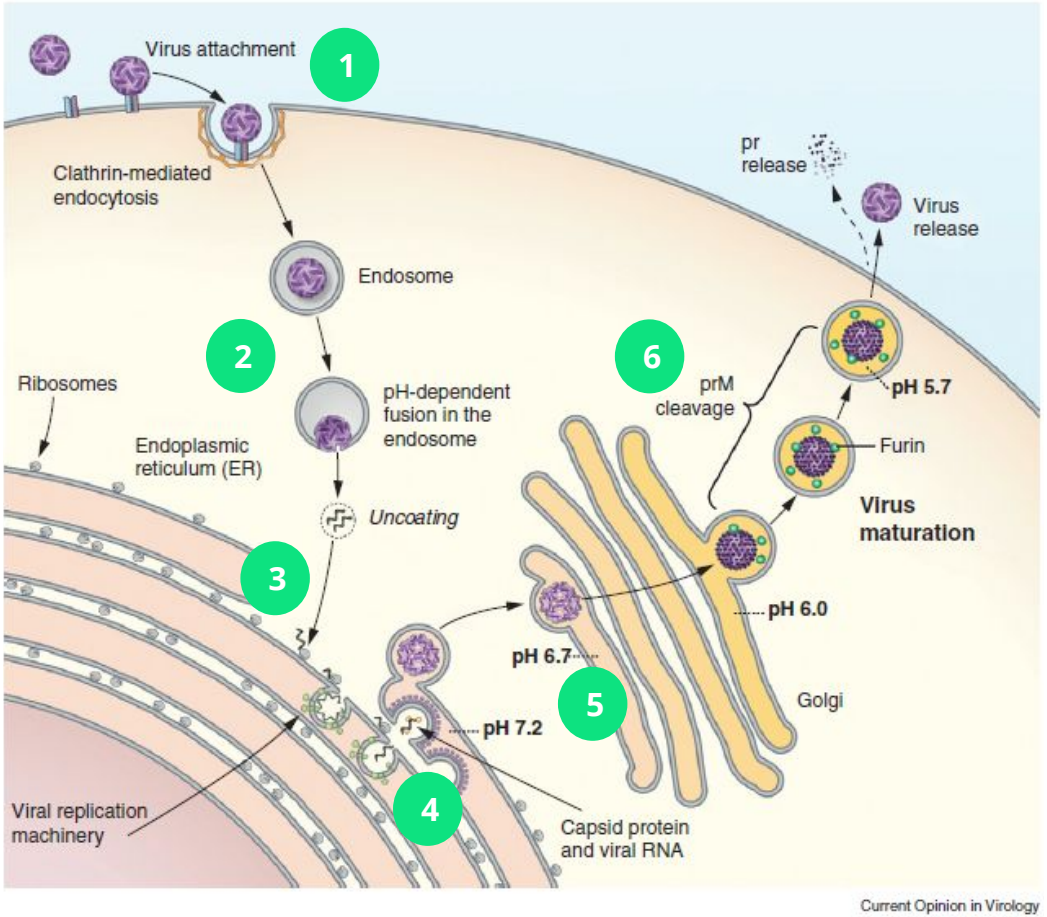
1. Attachment + Endocytosis
2. pH-dependent fusion
3. Releasement of RNA to the cytoplasm and entry to Endoplasmic Reticulum (ER)
4. Synthesis of viral proteins
5. Assembly of virions particles
6. Rearrangement - furin cleavage



Flavivirus infection cycle. Source: Pierson TC et al. (2012)

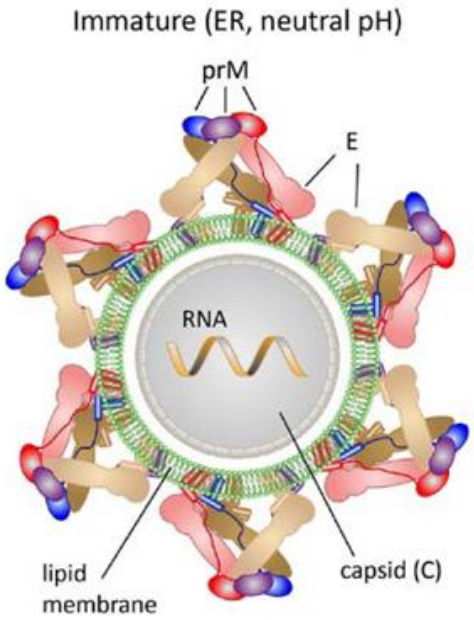
INFECTION CYCLE



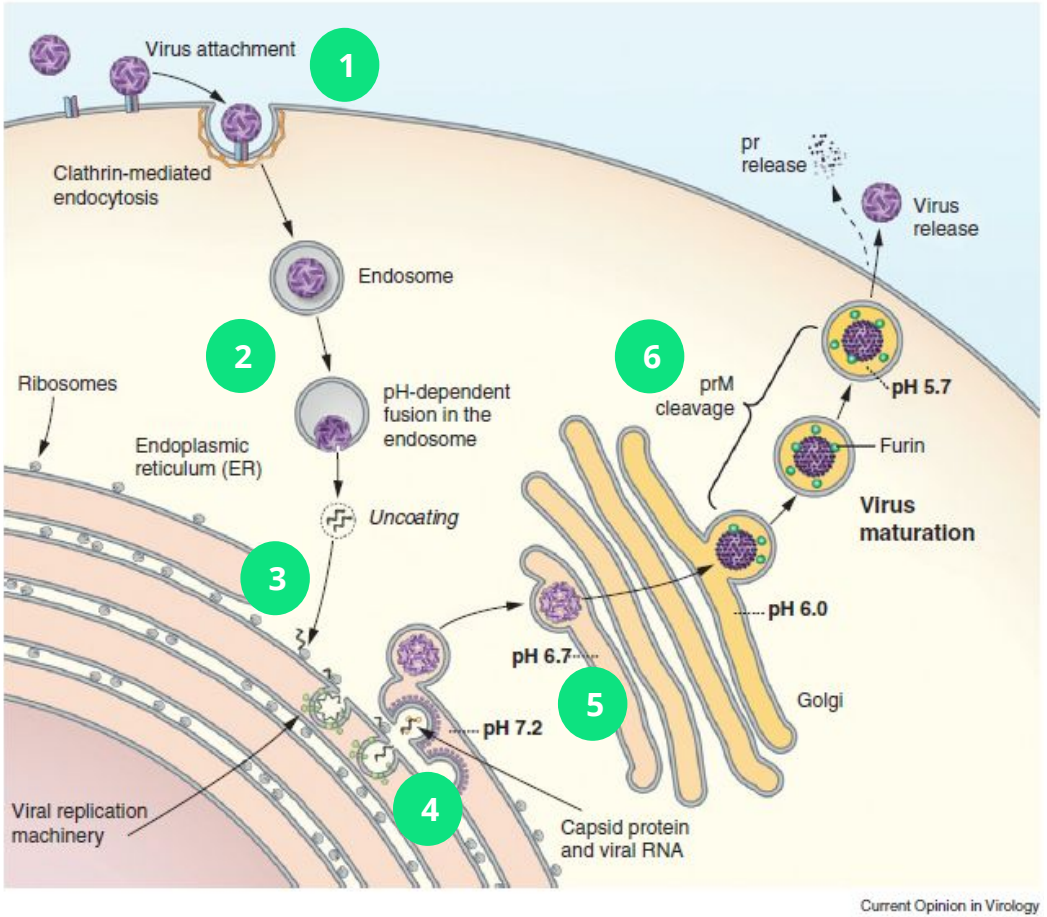


Flavivirus infection cycle. Source: Pierson TC et al. (2012)

INFECTION CYCLE

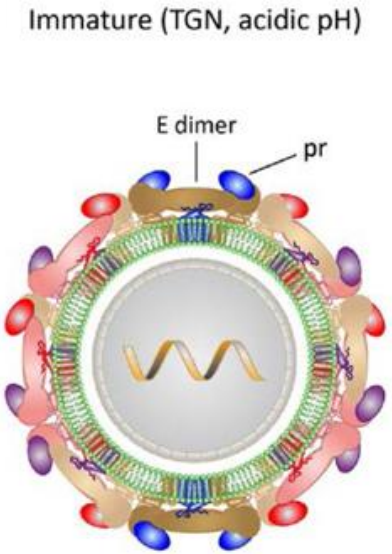


Modified from Rey F et al. (2017)



Flavivirus infection cycle. Source: Pierson TC et al. (2012)

INFECTION CYCLE



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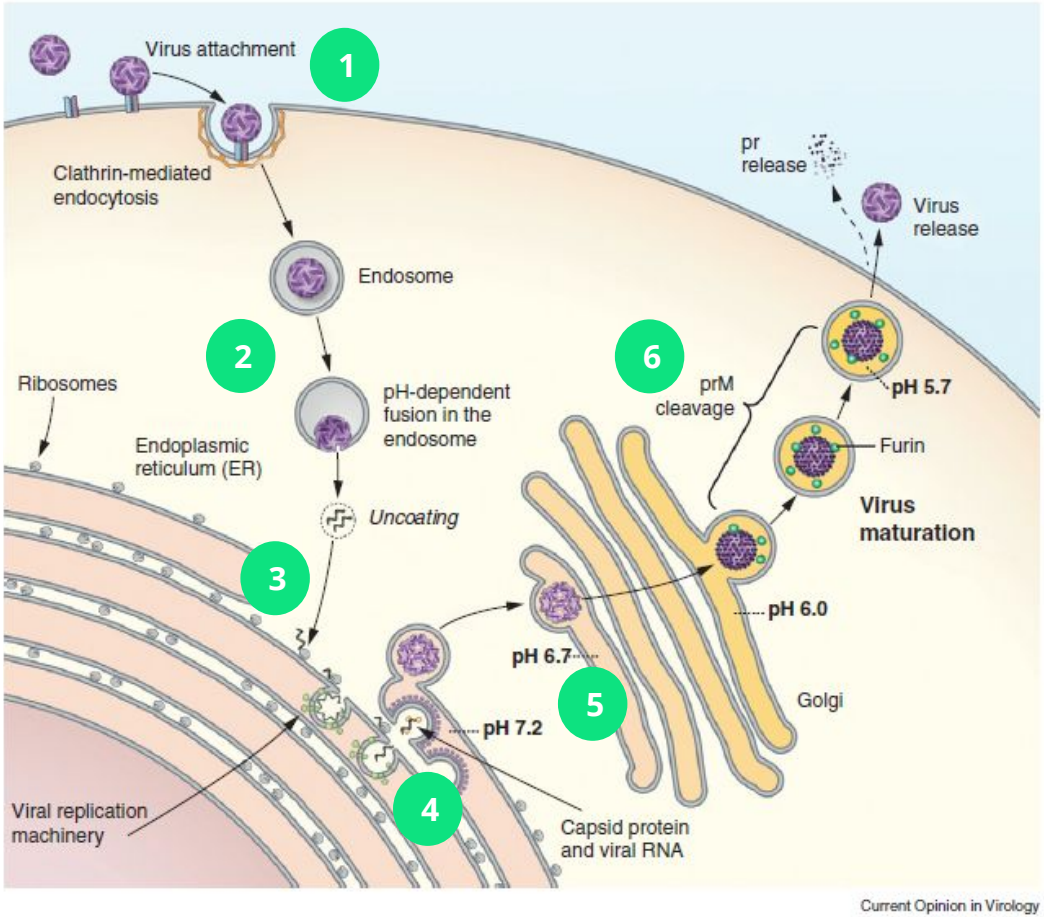
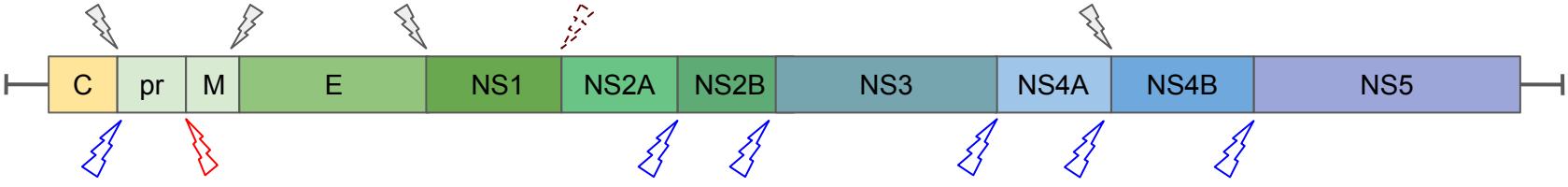
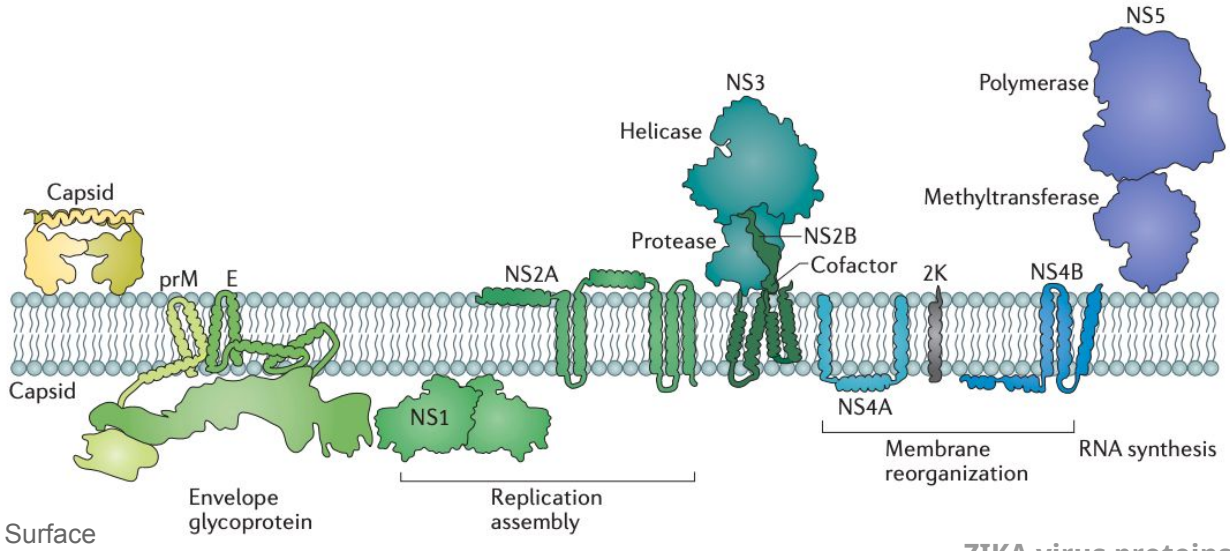


Flavivirus infection cycle. Source: Pierson TC et al. (2012)

INFECTION CYCLE

PROTEIN PROCESSING





Signal peptidase

Furin (TGN or Secretory granules)

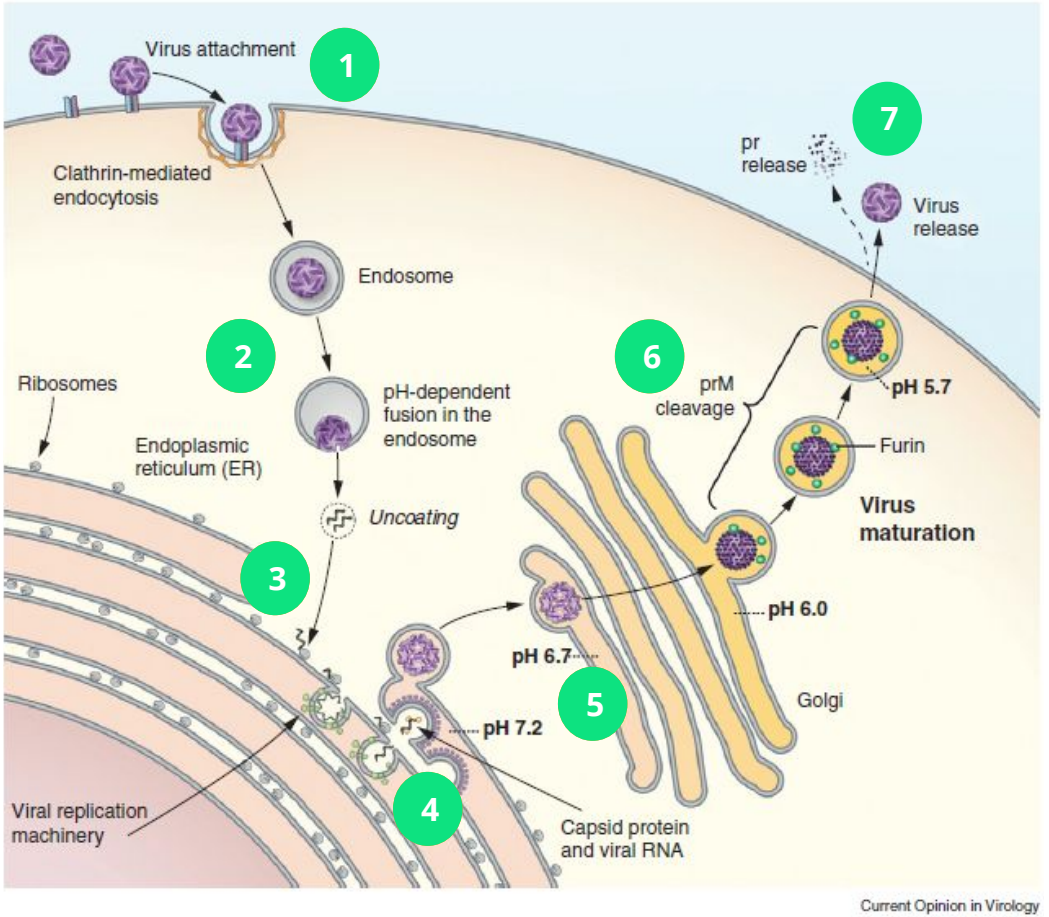
NS2B-3 protease (Cytoplasm)

Unknow (ER)

ZIKA virus proteins. Source: Christopher J, et al. (2018)

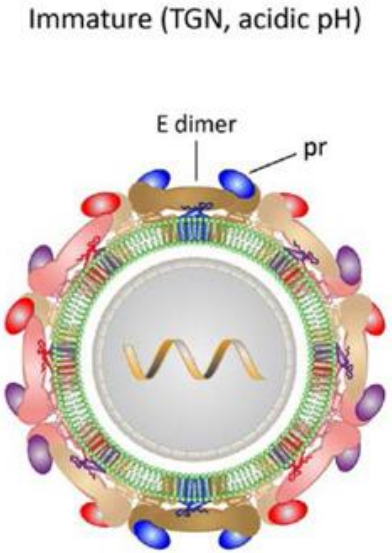
INFECTION CYCLE

1. Attachment + Endocytosis
2. pH-dependent fusion
3. Releasement of RNA to the cytoplasm and entry to Endoplasmic Reticulum (ER)
4. Synthesis of viral proteins
5. Assembly of virions particles
6. Rearrangement - furin cleavage
7. Pr domain releasement



Flavivirus infection cycle. Source: Pierson TC et al. (2012)

INFECTION CYCLE



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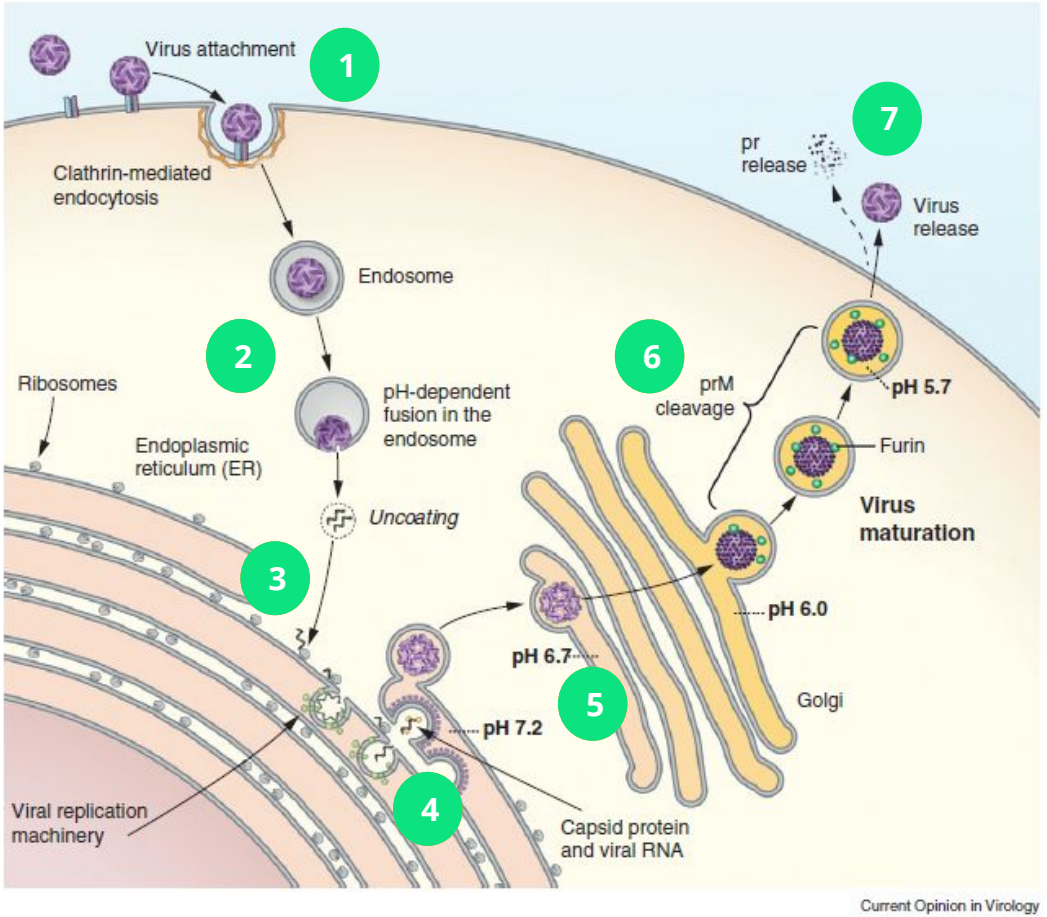
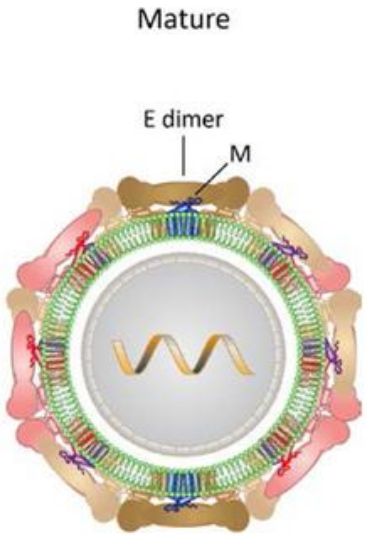


INFECTION CYCLE



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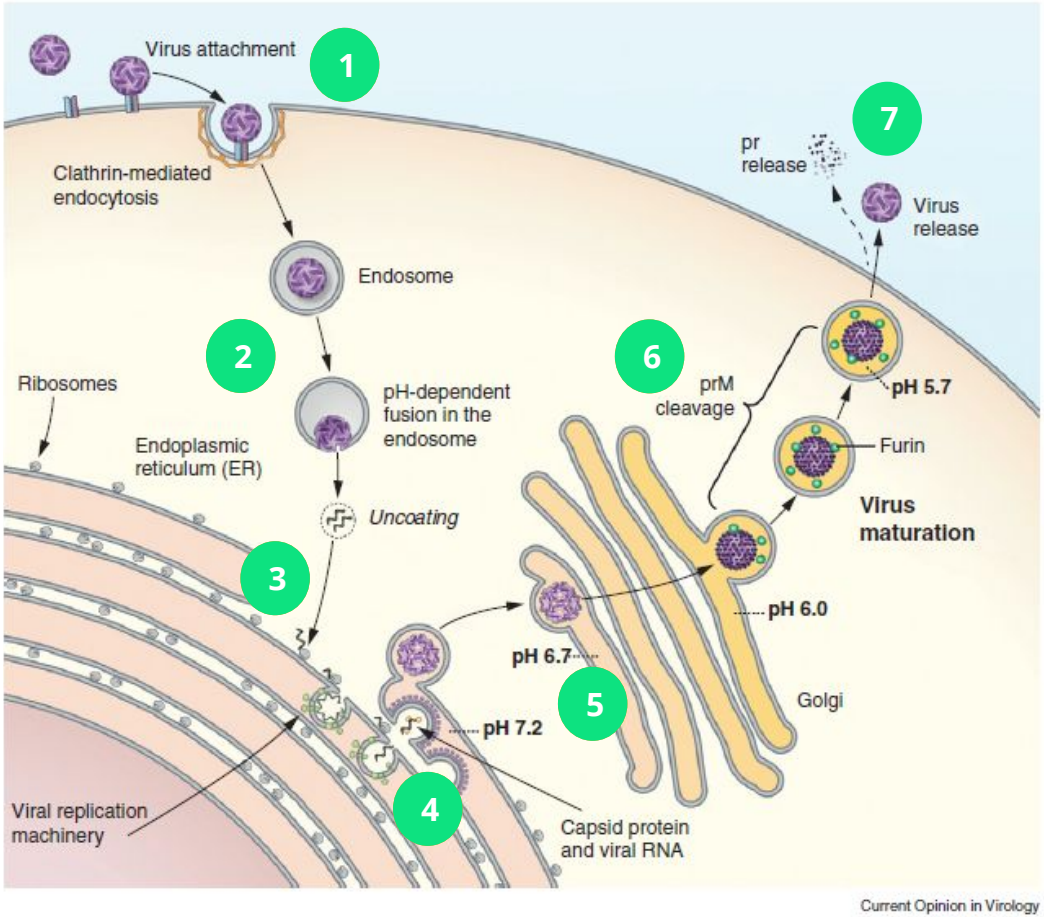
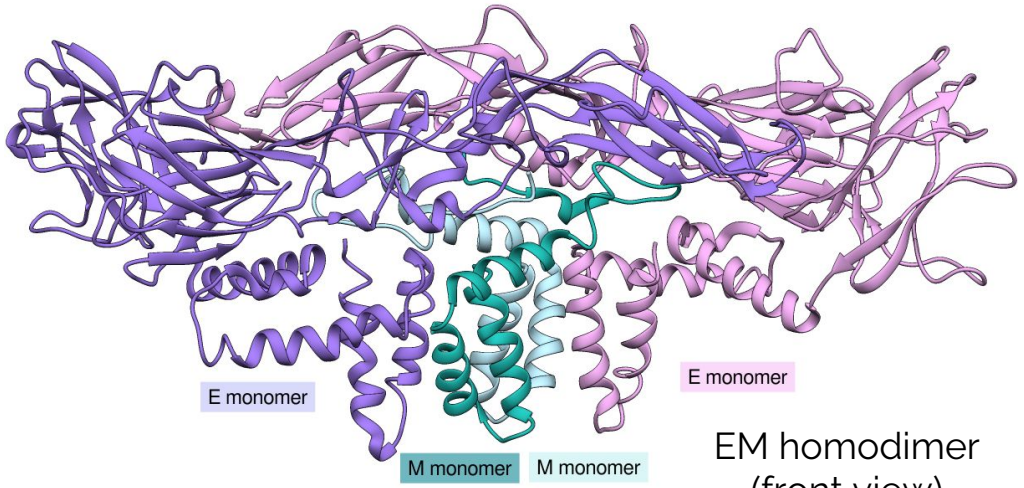


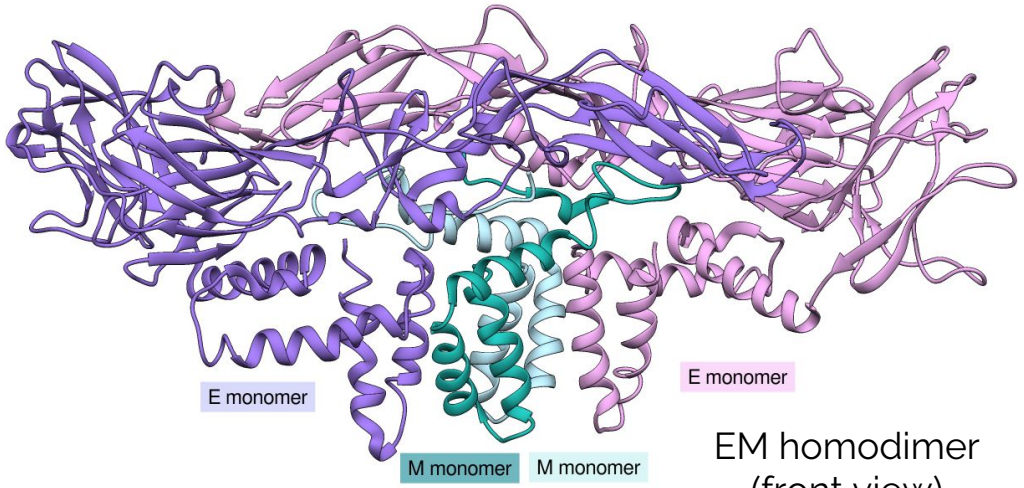
Envelope elements



EM homodimer
(front view)

Envelope elements

E protein

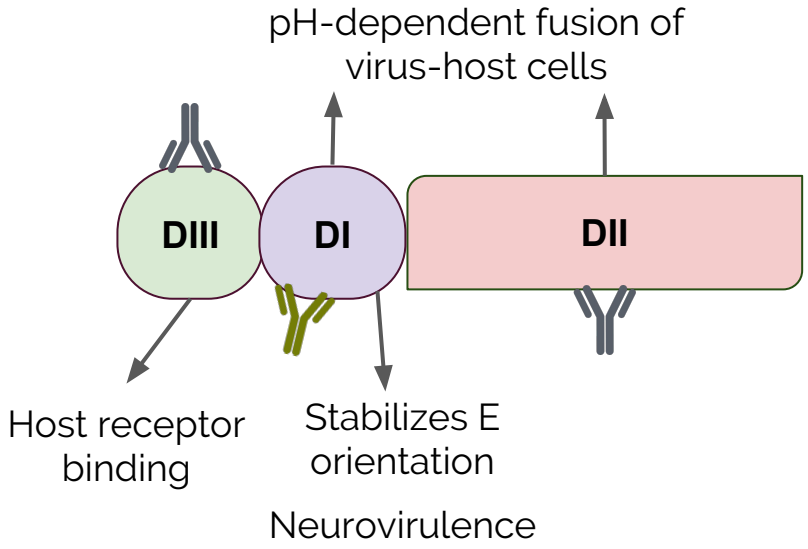


EM homodimer
(front view)

E PROTEIN: MAIN FEATURES

Important role in

- Cell attachment
- Membrane fusion
- Cell tropism
- Assembly and maturation
- Antigenicity

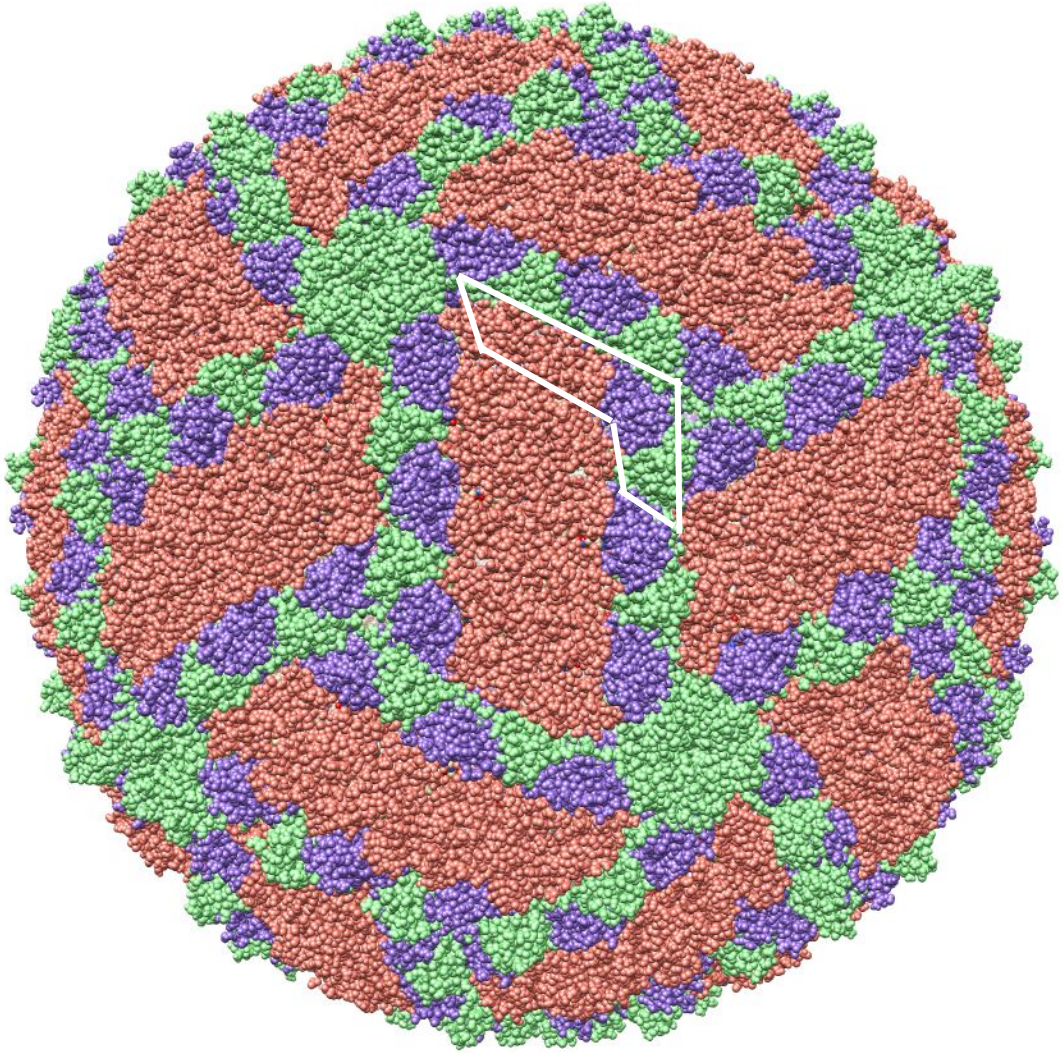


Main target of antibodies

Neutralizing epitopes

Non-neutralizing epitopes

E PROTEIN

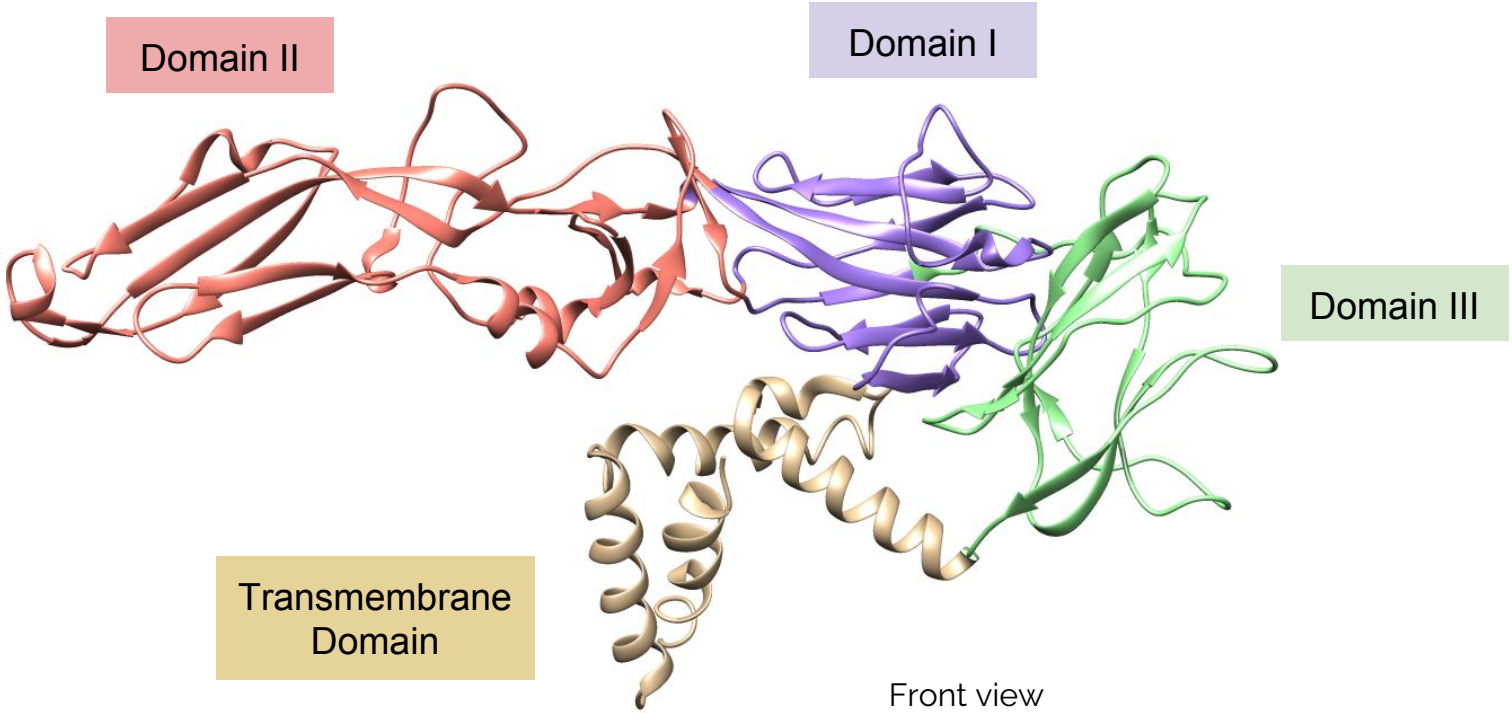


Domain I

Domain II

Domain III

E PROTEIN


Domain II
Domain I
Domain III
Transmembrane Domain
Front view

E PROTEIN: TOPOLOGICAL DIAGRAM

Domain I

- 9 β strands (A_0-I_0)
- 3_{10} helix

→ In the N-terminus

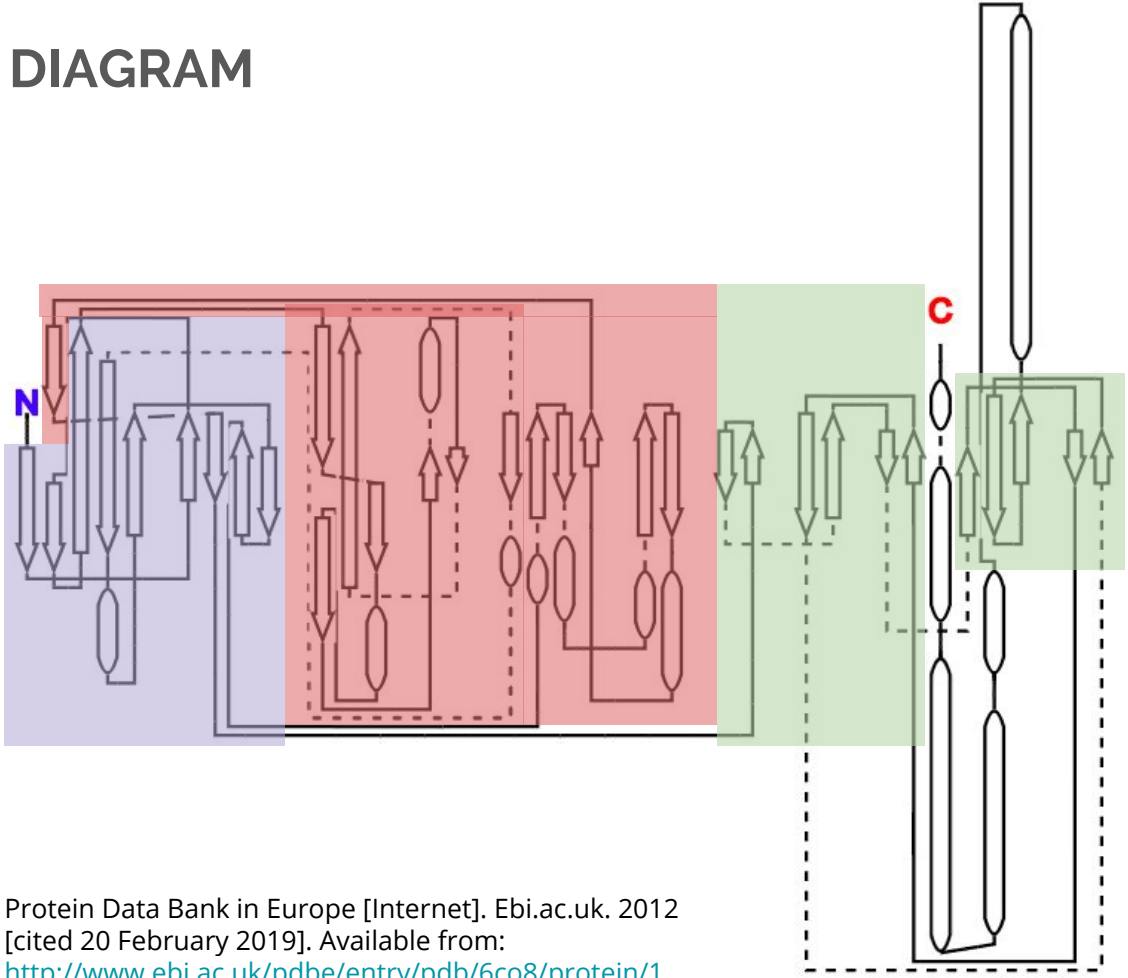
Domain II

- 9 β strands (a-i)
- 2 α helix (A,B)

Domain III

- 7 β strands (A-G)

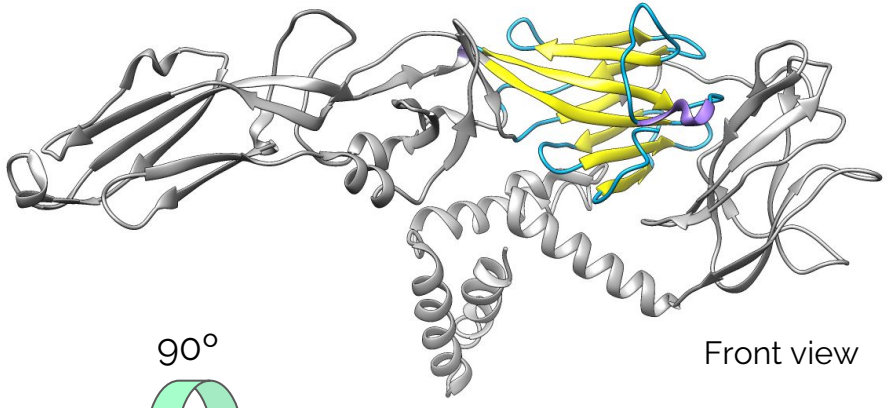
→ In the C-terminus

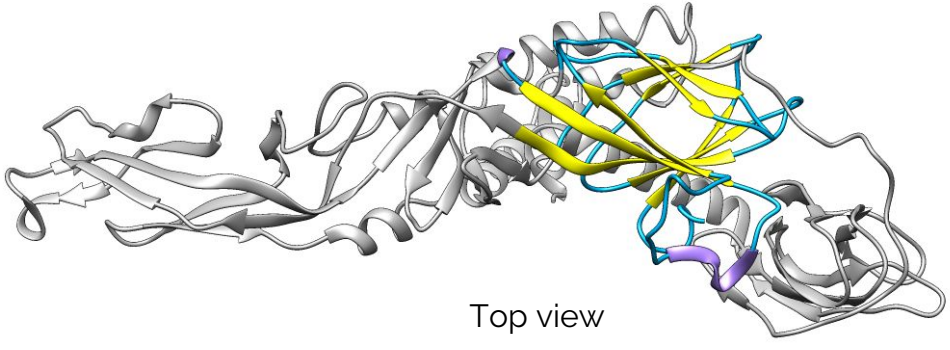


Protein Data Bank in Europe [Internet]. Ebi.ac.uk. 2012 [cited 20 February 2019]. Available from: http://www.ebi.ac.uk/pdbe/entry/pdb/6co8/protein/1

E PROTEIN: DOMAINS

DOMAIN I	
Level	
C	Mainly Beta
A	Sandwich
T	Tick-borne Encephalitis virus Glycoprotein; domain 1
H	Tick-borne Encephalitis virus Glycoprotein; domain 1

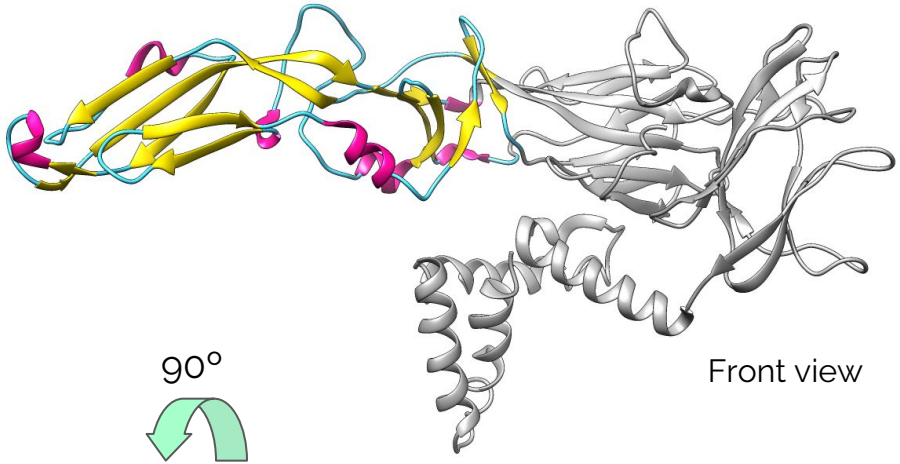


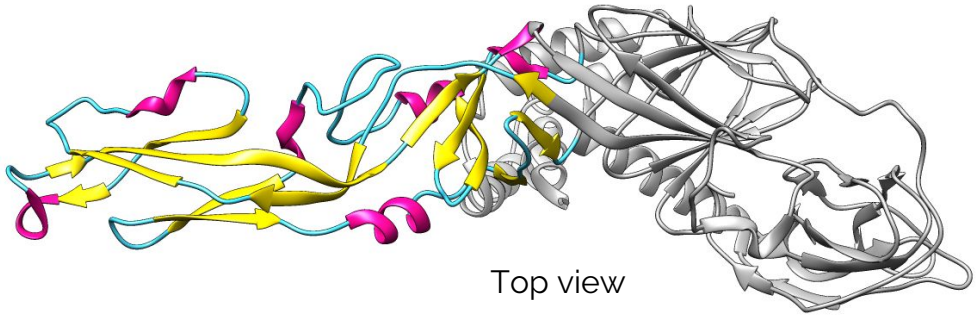


C lass
A rchitecture
T opology
H omologous Superfamily

E PROTEIN: DOMAINS

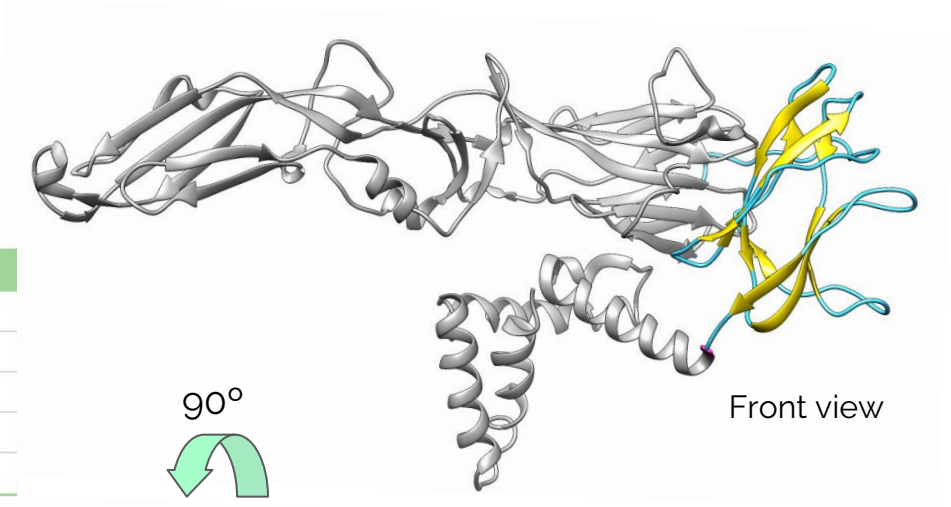
DOMAIN II	
Level	
C	Alpha Beta
A	2-Layer Sandwich
T	Viral Envelope Glycoprotein; domain 2
H	Viral Envelope Glycoprotein; domain 2

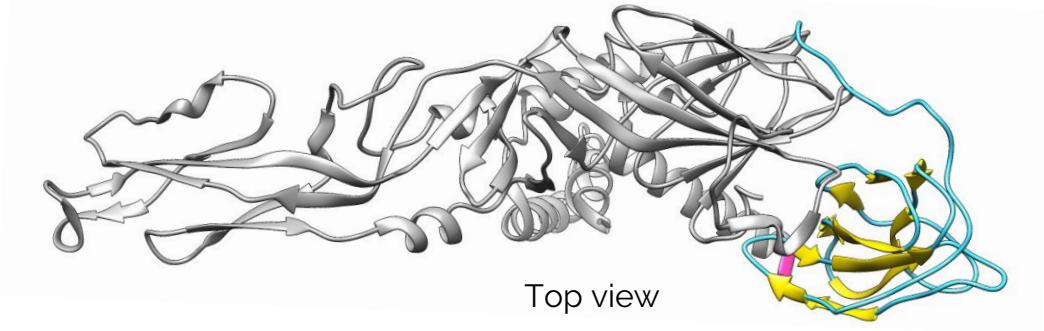




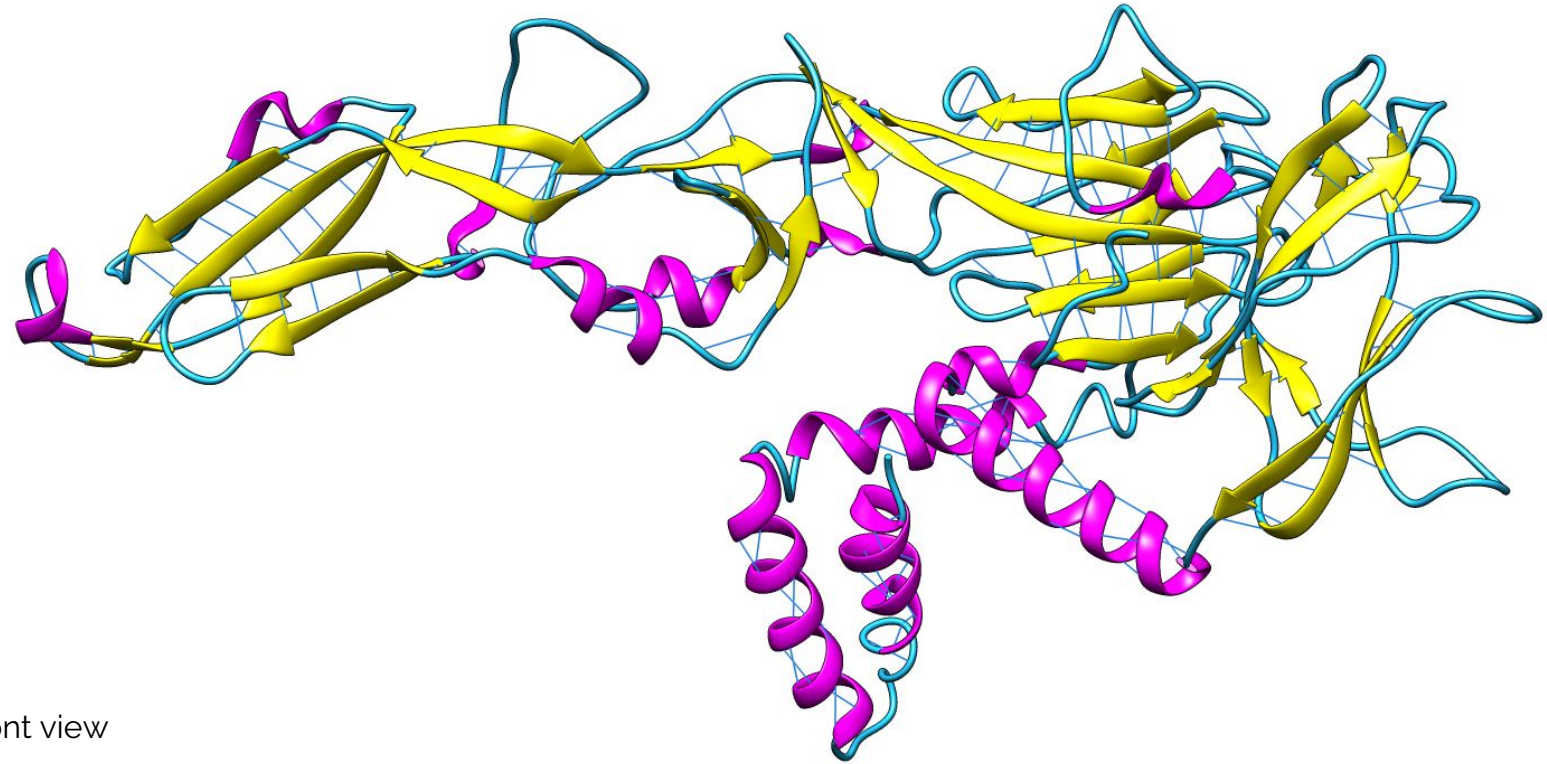
E PROTEIN: DOMAINS

DOMAIN III	
Level	
C	Alpha Beta
A	Sandwich
T	Immunoglobulin-like
H	



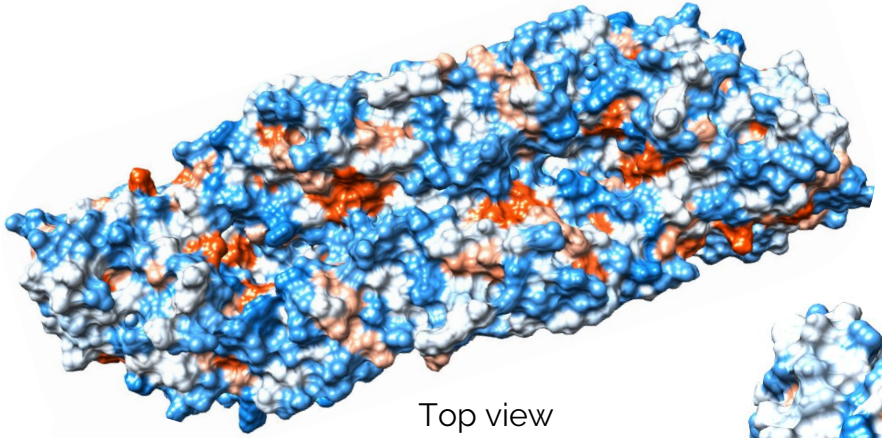


E PROTEIN: DOMAINS INTERACTIONS

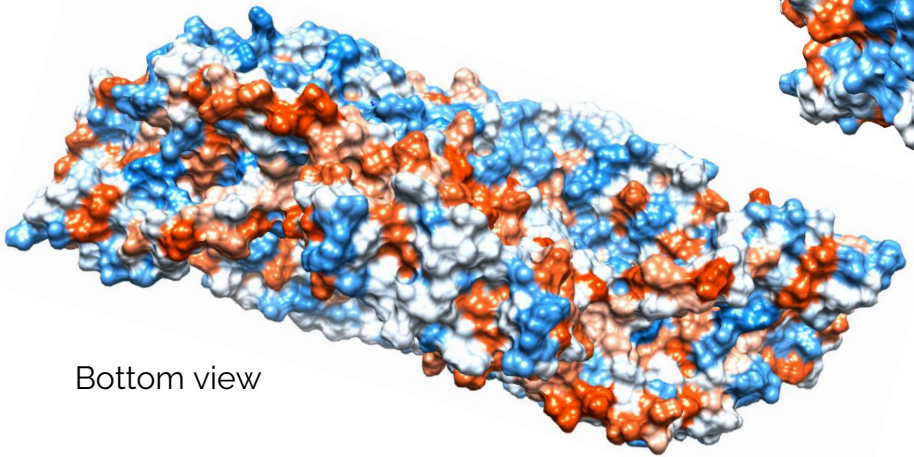


Front view

EXPLORING THE E SURFACE



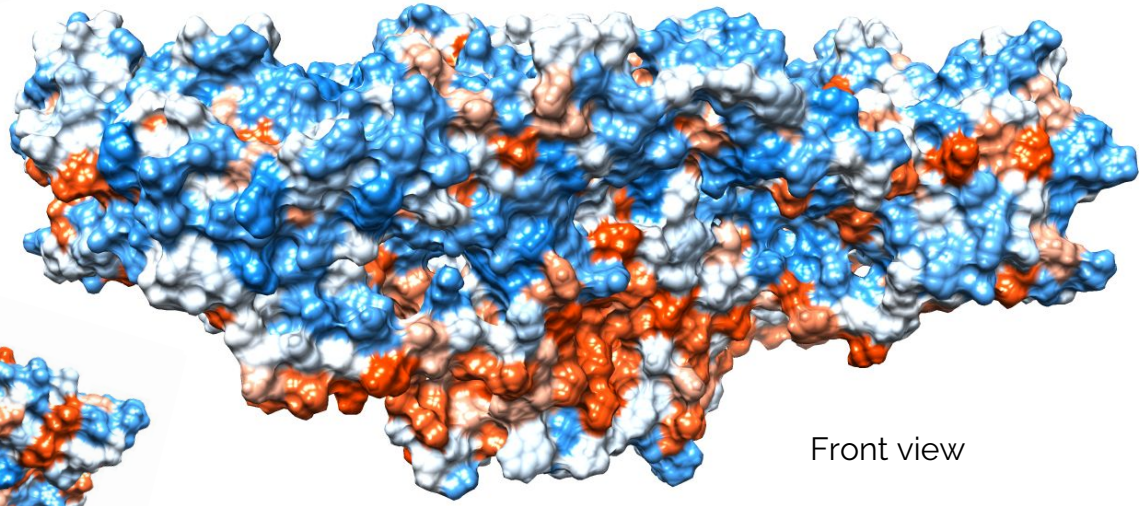
Top view



Bottom view

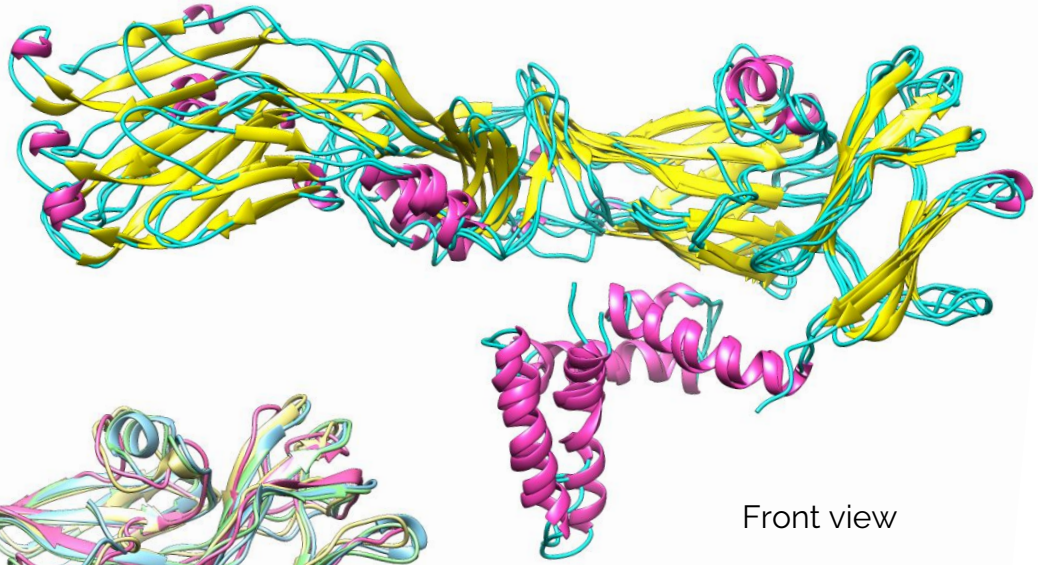
Hydrophobic regions

Hydrophilic regions

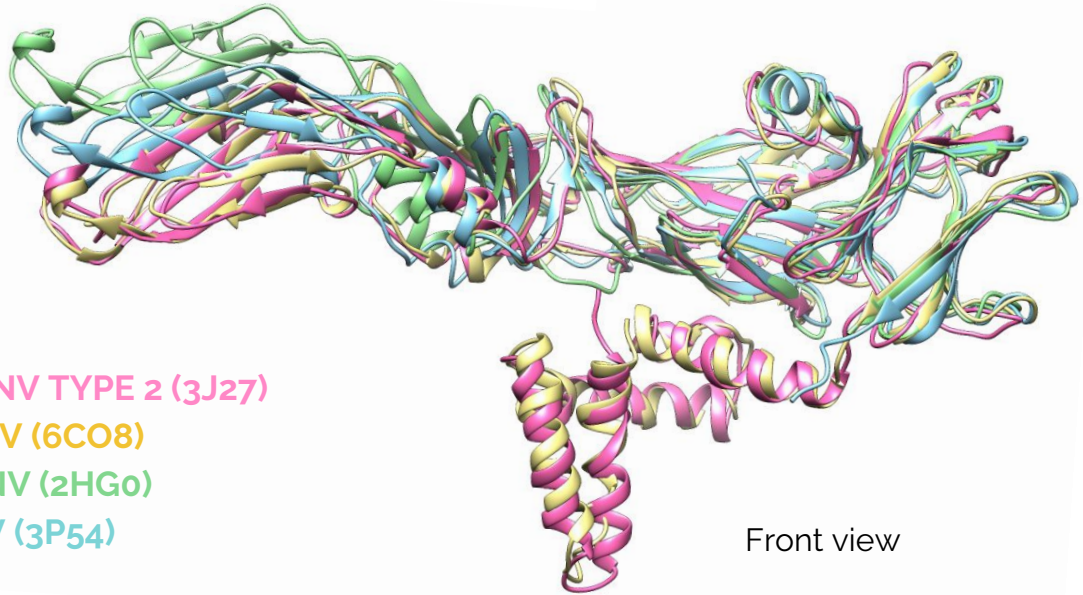


Front view

E PROTEIN: FLAVIVIRUSES SUPERIMPOSITION



Front view

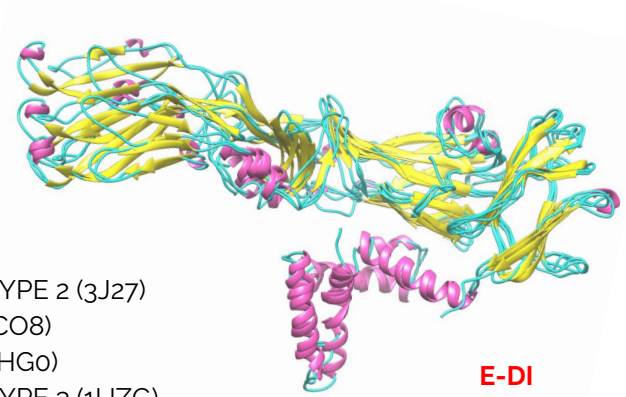


DENV TYPE 2 (3J27)
ZIKV (6CO8)
WNV (2HG0)
JEV (3P54)

Front view

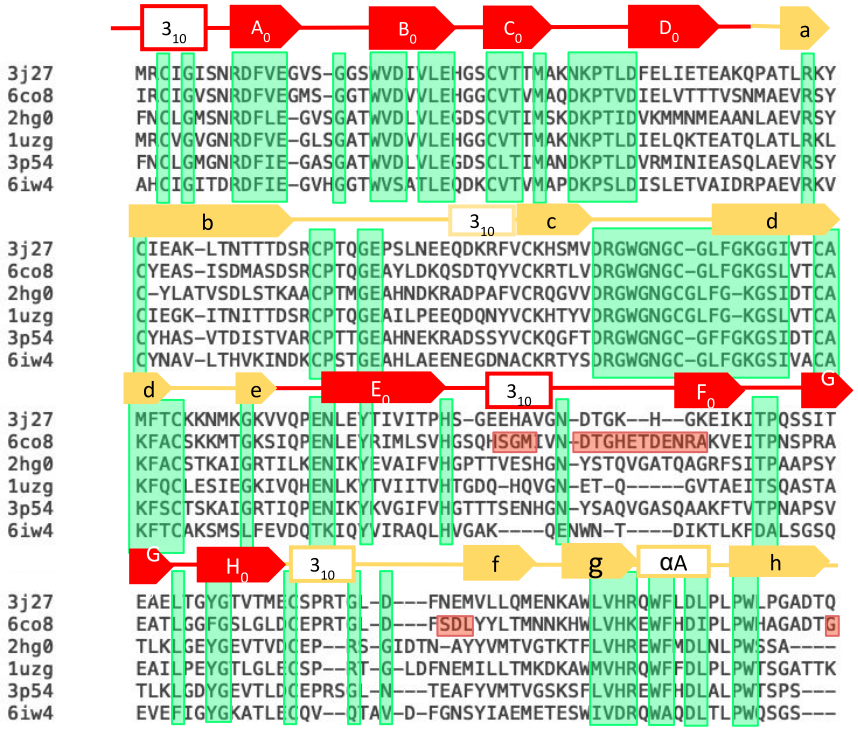
Score: **7,22**
RMSD: 2,51
Length:418
nfit: 359 (86%)

E PROTEIN: FLAVIVIRUSES SUPERIMPOSITION

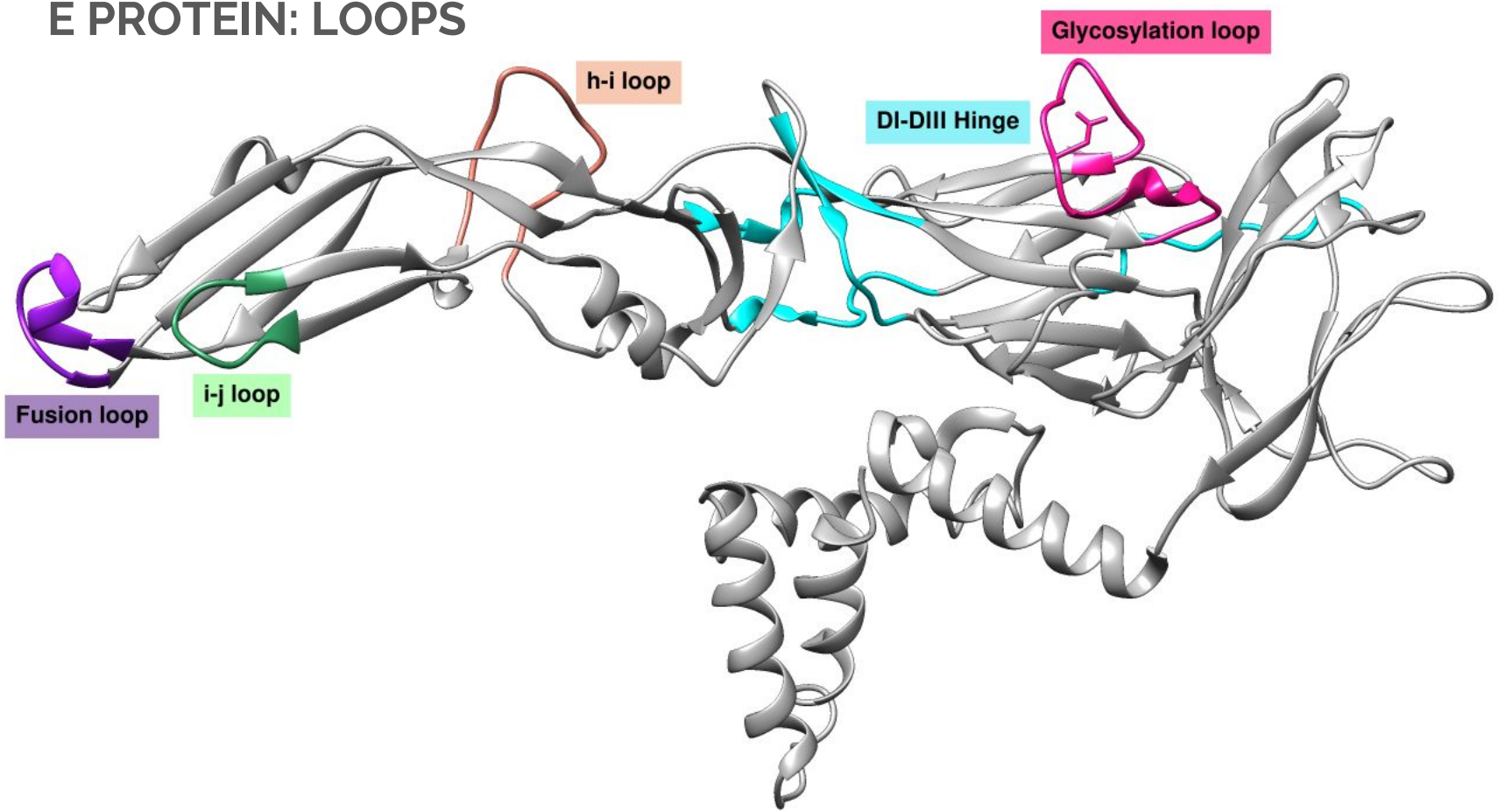


DENV TYPE 2 (3J27)
ZIKV (6CO8)
WNV (2HG0)
DENV TYPE 3 (1UZG)
JEV (3P54)
YFV (6IW4)

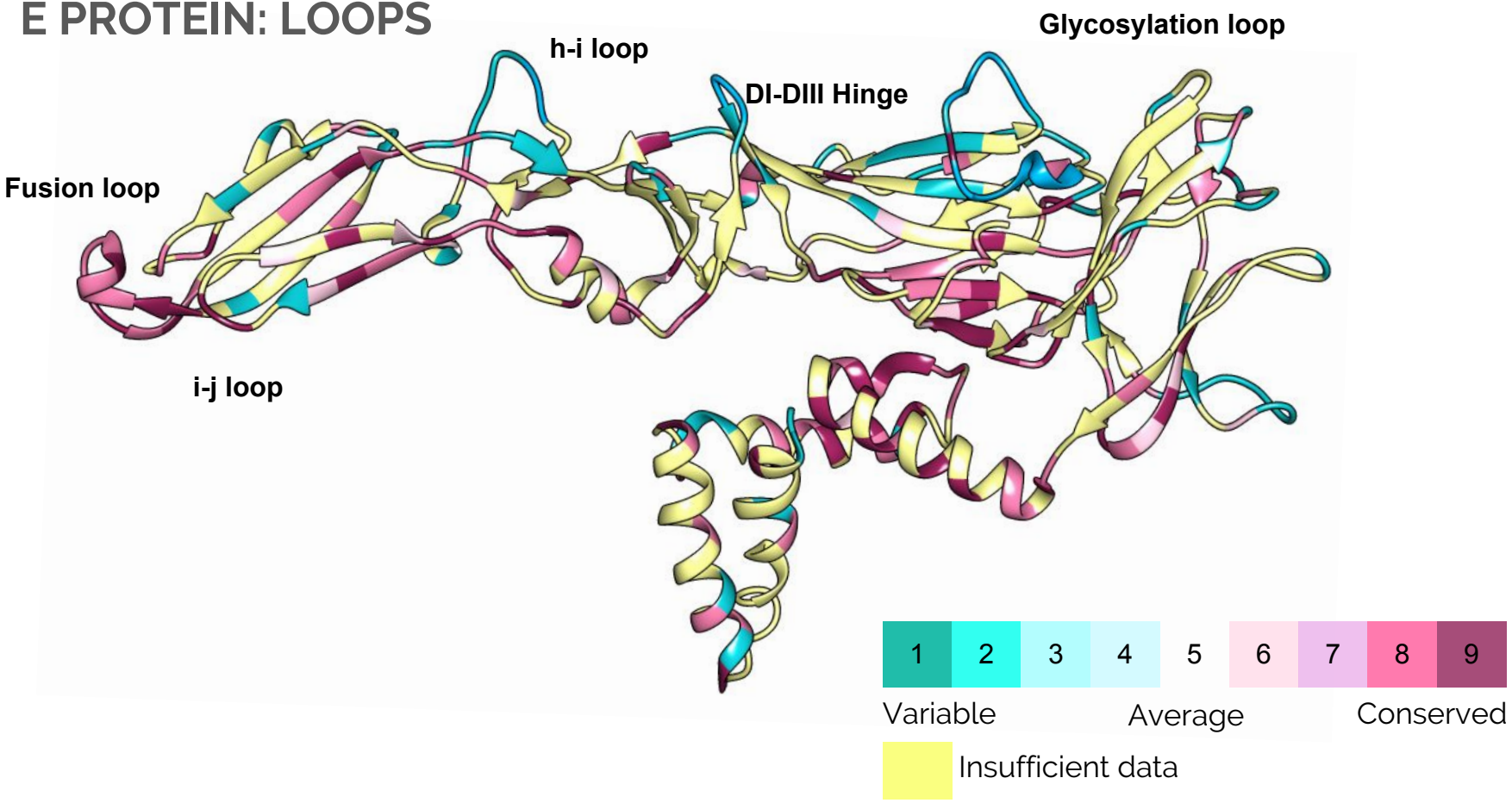
E-DI
E-DII
E-DIII



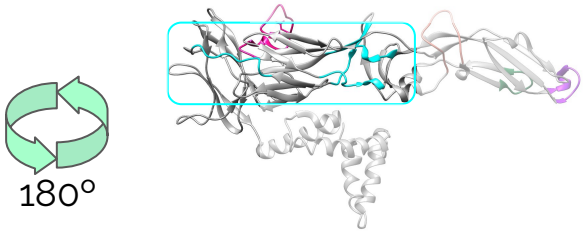
E PROTEIN: LOOPS



E PROTEIN: LOOPS



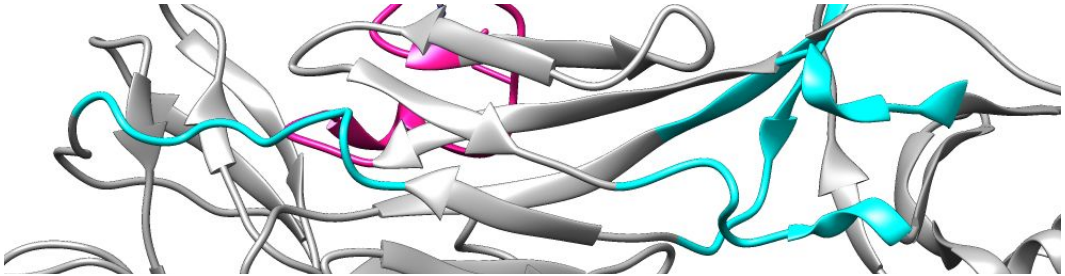
E PROTEIN: VARIABLE LOOPS



DI-DIII Hinge

Domain movement, transformation of dimer to the fusogenic trimer.

Back view



```
Zika_virus_1/1-504                      IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTV
Zika_virus_2/1-498                      IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTV
Japanese_encephalitis_virus_1/1-500     FNCLGMGNRDFIEGASGATWVDLVLEGDSCLTIMANDKPTLDVRMINIEA
Japanese_encephalitis_virus_2/1-499     -NCLGMGNRDFIEGASGATWVDLVLEGDSCLTIMANDKPTLDVRMINIEA
West_Nile_virus_1/1-501                 FNCLGMSNRDFLEGVSGATWVDLVLEGDSCVTIMSKDKPTIDVKMMNMEA
West_Nile_virus_2/1-501                 FNCLGMSNRDFLEGVSGATWVDLVLEGDSCVTIMSKDKPTIDVKMMNMEA
Dengue_virus_4_1/1-495                  MRCVGVGNRDFVEGVSGGAWVDLVLEHGGCVTTMAQGKPTLDFELTKTTA
Dengue_virus_4_2/1-495                  MRCVGVGNRDFVEGVSGGAWVDLVLEHGGCVTTMAQGRPTLDFELTKTTA
Dengue_virus_1_1/1-495                  MRCVGIGSRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTEV
Dengue_virus_1_2/1-495                  MRCVGIGNRDFVEGLSGATWVDVVLEHGSCVTTMAKNKPTLDIELLKTEV
Dengue_virus_3_1/1-493                  MRCVGVGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELQKTEA
Dengue_virus_3_2/1-493                  MRCVGVGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELQKTEA
Dengue_virus_2_1/1-495                  MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEA
Dengue_virus_2_2/1-495                  MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEA
Yellow_fever_virus_1/1-493              AHCIGITDRDFIEGVHGGTWVSATLEQDKCVTVMAPDKPSLDISLETVAI
Yellow_fever_virus_2/1-493              AHCIGITDRDFIEGVHGGTWVSATLEQDKCVTVMAPDKPSLDISLETVAI
                                         .*:*: .***:**  *.:**. .** . *:* *: .:*::*. : .

Zika_virus_1/1-504                      WGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG 150
Zika_virus_2/1-498                      WGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG 150
Japanese_encephalitis_virus_1/1-500     WGNGCGLFGKGSIDTCAKFSCTSKAIGRTIQPENLKYEVGIFVHGTTTSE 150
Japanese_encephalitis_virus_2/1-499     WGNGCGLFGKGSIDTCAKFSCTSKAIGRTIQPENLKYEVGIFVHGTTTSE 149
West_Nile_virus_1/1-501                 WGNGCGLFGKGSIDTCAKFACSTNATGRTILKENLKYEVAIFVHGPTTVE 150
West_Nile_virus_2/1-501                 WGNGCGLFGKGSIDTCAKFACTTKATGWIIQKENLKYEVAIFVHGPTTVE 150
Dengue_virus_4_1/1-495                  WGNGCGLFGKGGVVTCAKFSCSGKITGNLVQIENLEYTVVVTVHNGDTH- 149
Dengue_virus_4_2/1-495                  WGNGCGLFGKGGVVTCAKFSCSGKITGNLVQIENLEYTVVVTVHNGDTH- 149
Dengue_virus_1_1/1-495                  WGNGCGLFGKGSLITCAKFKCVTKLEGKIVQYENLKYSVIVTVHTGDQH- 149
Dengue_virus_1_2/1-495                  WGNGCGLFGKGSLLTCAKFKCVTKLEGKIVQYENLKYSVIVTVHTGDQH- 149
Dengue_virus_3_1/1-493                  WGNGCGLFGKGSLVTCAKFQCLELIEGKVVQHENLKYTVIITVHTGDQH- 149
Dengue_virus_3_2/1-493                  WGNGCGLFGKGSLVTCAKFQCLESIEGKVVQHENLKYTVTITVHTGDQH- 149
Dengue_virus_2_1/1-495                  WGNGCGLFGKGGIVTCAMFTCKKNMEGKIVQPENLEYTIVVTPHSGEEH- 149
Dengue_virus_2_2/1-495                  WGNGCGLFGKGGIVTCAMFTCKKNMEGKVVQPENLEYTIVITPHSGEEH- 149
Yellow_fever_virus_1/1-493              WGNGCGLFGKGSIVACAKFTCAKSMSLFEVDQTKIQYVIRAQLHVGAKQE 150
Yellow_fever_virus_2/1-493              WGNGCGLFGKGSIVACAKFTCAKSMSLFEVDQTKIQYVIRAQLHVGAKQE 150
                                        **********.: :** * *       :   : :* :    *
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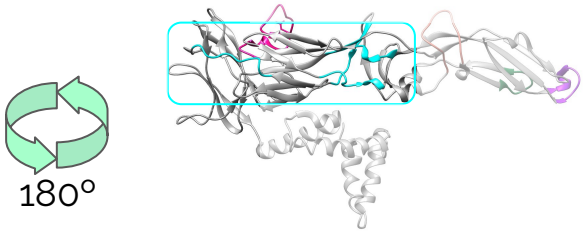
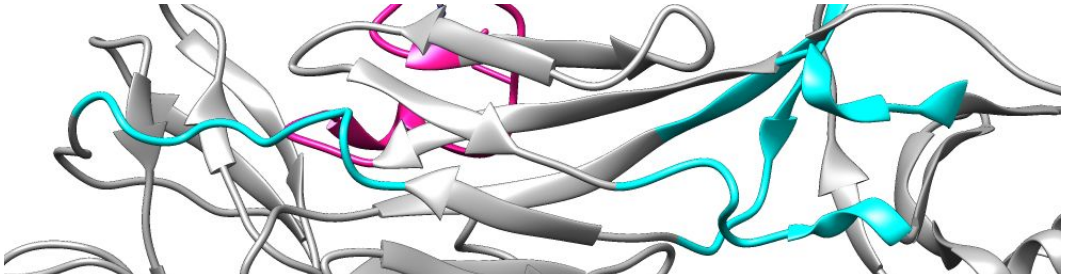
E PROTEIN: VARIABLE LOOPS



DI-DIII Hinge

Domain movement, transformation of dimer to the fusogenic trimer.

Back view



```
Zika_virus_1/1-504                      MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC:PRTGLDFSD
Zika_virus_2/1-498                      MIVND------ENRAKVEVTPNSPRAEATLGGFGSLGLDC:PRTGLDFSD
Japanese_encephalitis_virus_1/1-500     NHGNYSAQVGASQAAKFTVTPNAPSITLKLGDYGEVTLDC:PRSGLNTEA
Japanese_encephalitis_virus_2/1-499     NHGNYSAQVGASQAAKFTVTPNAPSITLKLGDYGEVTLDC:PRSGLNTEA
West_Nile_virus_1/1-501                 SHGNYSTQTGATQAGRFGITPAAPSYTLKLGEYGEVTVDC:PRSGIDTNA
West_Nile_virus_2/1-501                 SHGNYSTQMGATQAGRFSITPSAPSYTLKLGEYGEVTVDC:PRSGIDTSA
Dengue_virus_4_1/1-495                  AVGNDTS----NHGVTATITPRSPSVEVKLPDYGELTLDC:PRSGIDFNE
Dengue_virus_4_2/1-495                  AVGNDTS----NHGVTATITPRSPSVEVELPDYGELTLDC:PRSGIDFNE
Dengue_virus_1_1/1-495                  QVGNEST----EHGTTATITPQAPTSEIQLTDYGALTLDC:PRTGLDFNE
Dengue_virus_1_2/1-495                  QVGNETT----EHGTIATITPQAPTSEIQLTDYGALTLDC:PRTGLDFNE
Dengue_virus_3_1/1-493                  QVGNET------QGVTAEITPQASTVEAILPEYGTLGLEC:PRTGLDFNE
Dengue_virus_3_2/1-493                  QVGNDT------QGVTAEITPQASTVEAILPEYGTLGLEC:PRTGLDFNE
Dengue_virus_2_1/1-495                  AVGNDTG----KHGKEIKVTPQSSITEAELTGYGTVTMEC:PRTGLDFNE
Dengue_virus_2_2/1-495                  AVGNDTG----KHGKEIKITPQSSITEAELTGYGTVTMEC:PRTGLDFNE
Yellow_fever_virus_1/1-493              NW-NTDIK-------TLKFDALSGSQEAEFTGYGRATLEC:QVQTAVDFS
Yellow_fever_virus_2/1-493              NW-NTDIK-------TLKFDALSGSQEAEFTGYGRATLEC:QVQTAVDFS
                                           *            . . :     :  :*   ::*  ::.:: .
```

```
KRQTVVVLGSQEGAVHTALAGALEAEMDGA---KGRLSSGHLKCRLKMDK
KRQTVVVLGSQEGAVHTALAGALEAEMDGA---KGRLFSGHLKCRLKMDK
TKQSVVALGSQEGGLHQALAGAIVVEYSS----SVKLTSGHLKCRLKMDK
TKQSVVALGSQEGSLHQALAGAIVVEYSS----SVKLTSGHLKCRLKMDK
TKQSVIALGSQEGALHQALAGAIPVEFSSN---TVKLTSGHLKCRVKLEK
TKQSVVALGSQEGALHQALAGAIPVEFSSN---TVKLTSGHLKCRVKMEK
KRQDVTVLGSQEGAMHSALAGATEVDSGD----GNHMFAGHLKCKVRMEK
KRQDVTVLGSQEGAMHSALAGATEVDSGD----GNHMFAGHLKCKVRMEK
KKQEVVVLGSQEGAMHTALTGATEIQTSG----TTTIFAGHLKCRLKMDK
KKQEVVVLGSQEGAMHTALTGATEIQTSG----TTTIFAGHLKCRLKMDK
KKQEVVVLGSQEGAMHTALTGATEIQTSG----GTSIFAGHLKCRLKMDK
KKQEVVVLGSQEGAMHTALTGATEIQNSG----GTSIFAGHLKCRLKMDK
KKQDVVVLGSQEGAMHTALTGATEIQMSS----GNLLFTGHLKCRLRMDK
KKQDVVVLGSQEGAMHTALTGATEIQMSS----GNLLFTGHLKCRLRMDK
ATIKVLALGNQEGSLKTALTGAMRVTKDTNNSKLYKLHGGHVACRVKLSA
ATIKVLALGNQEGSLKTALTGAMRVTKDTNNSKLYKLHGGHVACRVKLSA
   * .**.***.:: **:**     .  .     :  **: ':::::.
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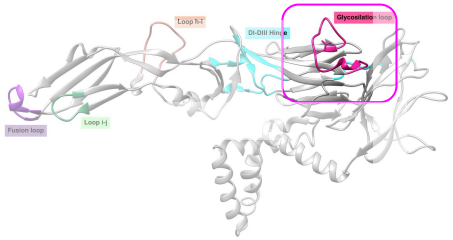
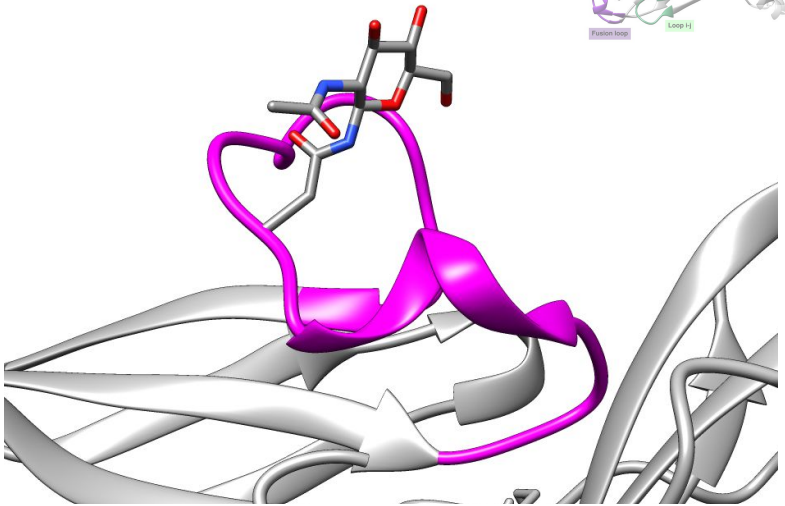
E PROTEIN: VARIABLE LOOPS



Glycosylation Loop

Related to ZIKA virulence pathogenesis and neuroinvasiveness



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Zika_virus_1/1-504                    WGNGCGLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG MIVNDTGHETDENRAKVEITPNSPRAEATLG
Zika_virus_2/1-498                    WGNGCGLFGKGSLVTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG MIVND------ENRAKVEVTPNSPRAEATLG
Japanese_encephalitis_virus_1/1-500   WGNGCGLFGKGSIDTCAKFSCTSKAIGRTIQPENIKYEVGIFVHGTTTSE NHGNYSAQVGASQAAKFTVTPNAPSITLKLG
Japanese_encephalitis_virus_2/1-499   WGNGCGLFGKGSIDTCAKFSCTSKAIGRTIQPENIKYEVGIFVHGTTTSE NHGNYSAQVGASQAAKFTVTPNAPSITLKLG
West_Nile_virus_1/1-501               WGNGCGLFGKGSIDTCAKFACSTNATGRTILKENIKYEVAIFVHGPTTVE SHGNYSTQTGATQAGRFGITPAAPSYTLKLG
West_Nile_virus_2/1-501               WGNGCGLFGKGSIDTCAKFACTTKATGWIIQKENIKYEVAIFVHGPTTVE SHGNYSTQMGATQAGRFSITPSAPSYTLKLG
Dengue_virus_4_1/1-495                WGNGCGLFGKGGVVTCAKFSCSGKITGNLVQIENLEYTVVVTVHNGDTH- AVGNDTS----NHGVTATITPRSPSVEVKLP
Dengue_virus_4_2/1-495                WGNGCGLFGKGGVVTCAKFSCSGKITGNLVQIENLEYTVVVTVHNGDTH- AVGNDTS----NHGVTATITPRSPSVEVELP
Dengue_virus_1_1/1-495                WGNGCGLFGKGSLITCAKFKCVTKLEGKIVQYENLKYSVIVTVHTGDQH- QVGNEST----EHGTTATITPQAPTSEIQLT
Dengue_virus_1_2/1-495                WGNGCGLFGKGSLLTCAKFKCVTKLEGKIVQYENLKYSVIVTVHTGDQH- QVGNETT----EHGTIATITPQAPTSEIQLT
Dengue_virus_3_1/1-493                WGNGCGLFGKGSLVTCAKFQCLELIEGKVVQHENLKYTVIITVHTGDQH- QVGNET------QGVTAEITPQASTVEAILP
Dengue_virus_3_2/1-493                WGNGCGLFGKGSLVTCAKFQCLESIEGKVVQHENLKYTVTITVHTGDQH- QVGNDT------QGVTAEITPQASTVEAILP
Dengue_virus_2_1/1-495                WGNGCGLFGKGGIVTCAMFTCKKNMEGKIVQPENLEYTIVVTPHSGEEH- AVGNDTG----KHGKEIKVTPQSSITEAELT
Dengue_virus_2_2/1-495                WGNGCGLFGKGGIVTCAMFTCKKNMEGKVVQPENLEYTIVITPHSGEEH- AVGNDTG----KHGKEIKITPQSSITEAELT
Yellow_fever_virus_1/1-493            WGNGCGLFGKGSIVACAKFTCAKSMSLFEVDQTKIQYVIRAQLHVGAKQE NW-NTDIK-------TLKFDALSGSQEAEFT
Yellow_fever_virus_2/1-493            WGNGCGLFGKGSIVACAKFTCAKSMSLFEVDQTKIQYVIRAQLHVGAKQE NW-NTDIK-------TLKFDALSGSQEAEFT
                                      **********.: :** * *       :   :::* :    *            *              . . :      :
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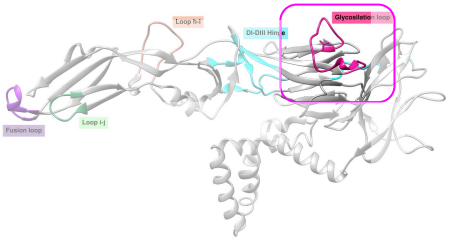
E PROTEIN: VARIABLE LOOPS



Glycosylation Loop

Asn 154 in ZIKV

Asn 153 in DENV

DENV TYPE 2 (3J27)

ZIKV (6CO8)

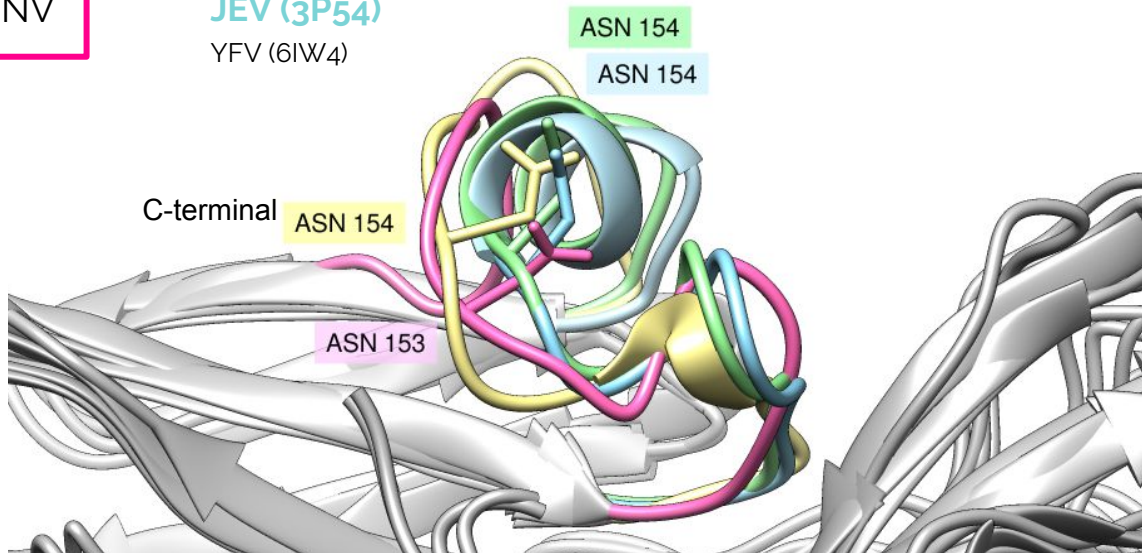
WNV (2HG0)

DENV TYPE 3 (1UZG)

JEV (3P54)

YFV (6IW4)

```
3j27    MFTCKKNMKGKVVQPENLEYTIVITPHS-GEEHAVGN-DTGK--H--GKEIKITPQSSIT
6co8    KFACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVN-DTGHETDENRAKVEITPNSPRA
2hg0    KFACSTKAIGRTILKENIKYEVAIFVHGPTTVESHGN-YSTQVGATQAGRFSITPAAPSY
1uzg    KFQCLESIEGKIVQHENLKYTVIITVHTGDQ-HQVGN-ETQ-----GVTAEITSQASTA
3p54    KFSCTSKAIGRTIQPENIKYKVGIFVHGTTTSENHGN-YSAQVGASQAAKFTVTPNAPSV
6iw4    KFTCAKSMSLFEVDQTKIQYVIRAQLHVGAK----QENWNTD-----IKTLKFDALSGSQ
```



E PROTEIN: VARIABLE LOOPS

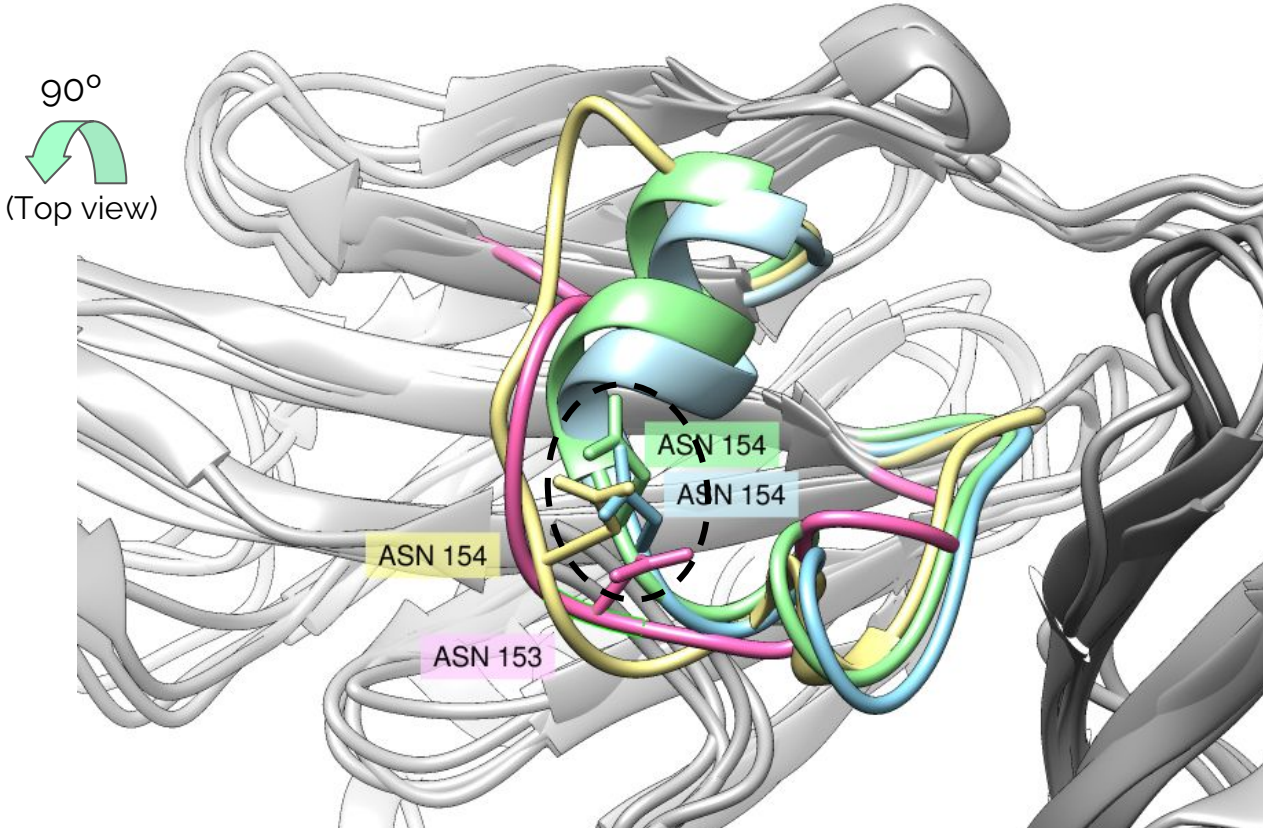
Glycosylation Loop

Asn 154 in ZIKV

Asn 153 in DENV

ZIKV (6CO8)
DENV TYPE 2 (3J27)
JEV (3P54)
WNV (2HG0)

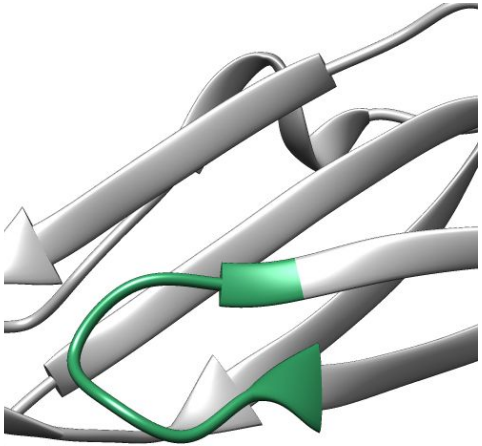
Conserved glycosylation site

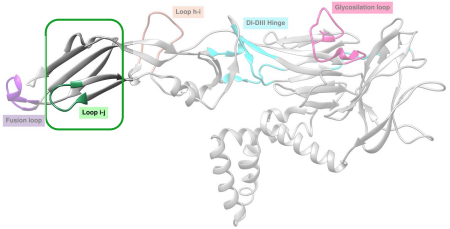


E PROTEIN: VARIABLE LOOPS

i-j loop

Related to **large conformational changes** during transformation from immature to mature forms.



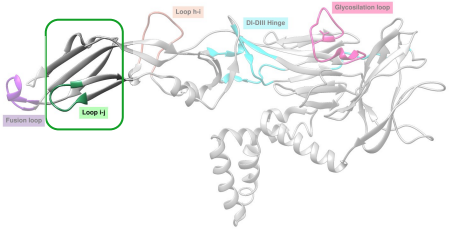


```
Zika_virus_1/1-504                      LYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGS
Zika_virus_2/1-498                      LYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGS
Japanese_encephalitis_virus_1/1-500     FYVMTVGSKSFLVHREWFHDLALPWTPPSST---AWRNRELLMEFEEAHATKQSVVALGS
Japanese_encephalitis_virus_2/1-499     FYVMTVGSKSFLVHREWFHDLALPWTSPSST---AWRNRELLMEFEEAHATKQSVVALGS
West_Nile_virus_1/1-501                 YYVMTVGTKTFLVHREWFMDLNLPWSSAGST---VWRNRETLMEFEEPHATKQSVIALGS
West_Nile_virus_2/1-501                 YYVMSVGAKSFLVHREWFMDLNLPWSSAGST---TWRNRETLMEFEEPHATKQSVVALGS
Dengue_virus_4_1/1-495                  MILMKMKKKTWLVHKQWFLDLPLPWTTGADTSEVHWNYKERMVTFKVPHAKRQDVTVLGS
Dengue_virus_4_2/1-495                  MILMKMNKKTWLVHKQWFLDLPLPWTAGADTSEVHWNNKERMVTFKVPHAKRQDVTVLGS
Dengue_virus_1_1/1-495                  MVLLTMKEKSWLVHKQWFLDLPLPWTSGASTSQETWNRQDLLVTFKTAHAKKQEVVVLGS
Dengue_virus_1_2/1-495                  MVLLTMKEKSWLVHKQWFLDLPLPWTSGASTSQETWNRQDLLVTFKTAHAKKQEVVVLGS
Dengue_virus_3_1/1-493                  MILLTMKNKAWMVHRQWFFDLPLPWTSGATTETPTWNKKELLVTFKNAHAKKQEVVVLGS
Dengue_virus_3_2/1-493                  MILLTMKNKAWMVHRQWFFDLPLPWTSGATTETPTWNRKELLVTFKNAHAKKQEVVVLGS
Dengue_virus_2_1/1-495                  MVLLQMENKAWLVHRQWFLDLPLPWLPGADKQESNWIQKETLVTFKNPHAKKQDVVVLGS
Dengue_virus_2_2/1-495                  MVLLQMEDKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTFKNPHAKKQDVVVLGS
Yellow_fever_virus_1/1-493              SYIAEMEKESWIVDKQWAQDLTLPWQSGSGG---VWREMHHLVEFEPPHAATIKVLALGN
Yellow_fever_virus_2/1-493              SYIAEMEKESWIVDKQWAQDLTLPWQSGSGG---VWREMHHLVEFEPPHAATIKVLALGN
                                              :  : ::*.::*  *: *** . .      *   . :: *  .**     * .**.
```

Larger differences between *Flaviviruses*

Differences in binding of **neutralizing antibodies.**

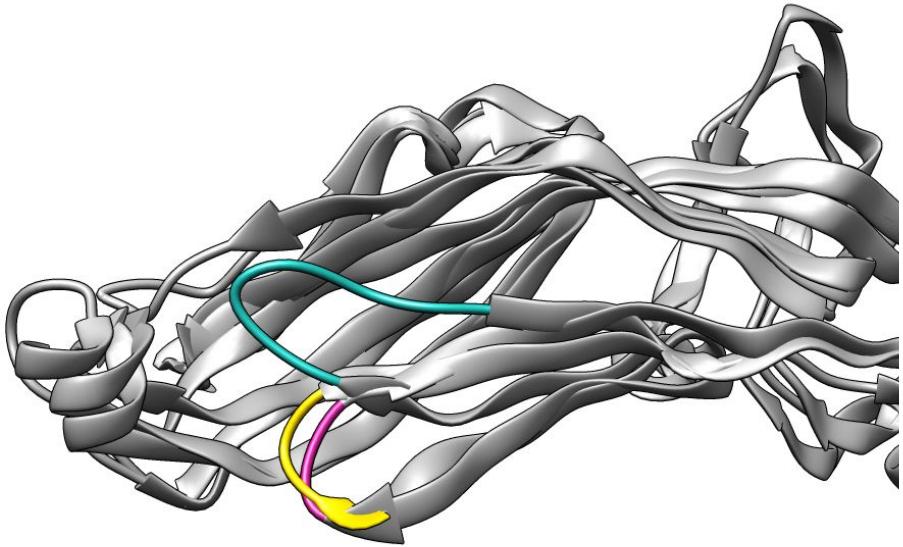
E PROTEIN: VARIABLE LOOPS



i-j loop

Similar closed conformation in **ZIKV** and **DENV**, whereas in **JEV** this loop has an open conformation.

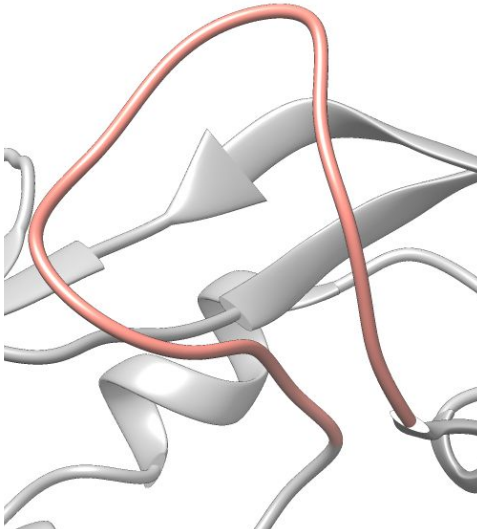
The differences in these loop conformations might account for differences in binding of neutralizing antibodies.

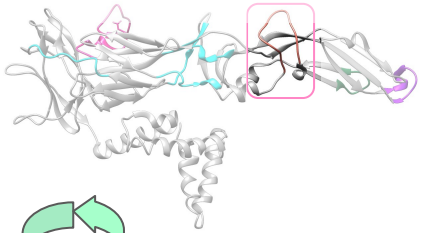


E PROTEIN: VARIABLE LOOPS

h-i loop

Related to **large conformational changes** during transformation from immature to mature forms.



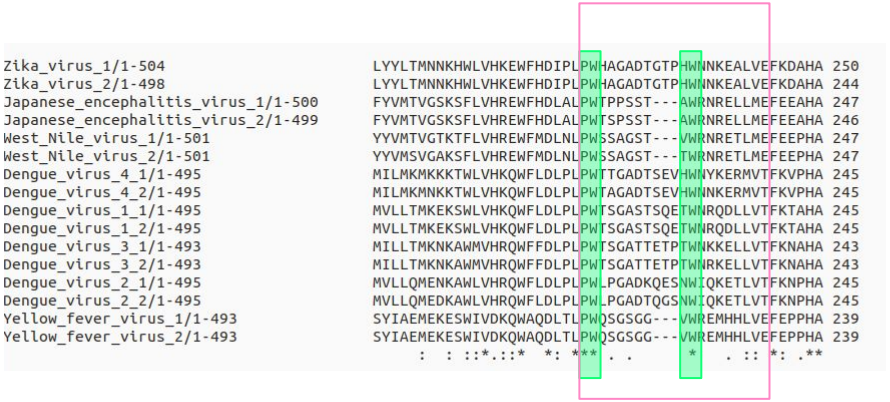


180°

Back view

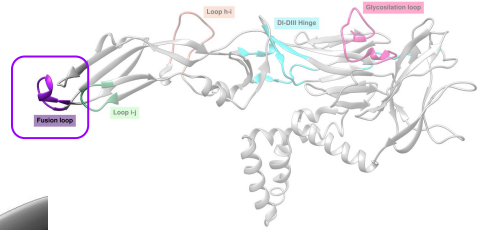
Larger differences between *Flaviviruses*

Differences in binding of **neutralizing antibodies.**



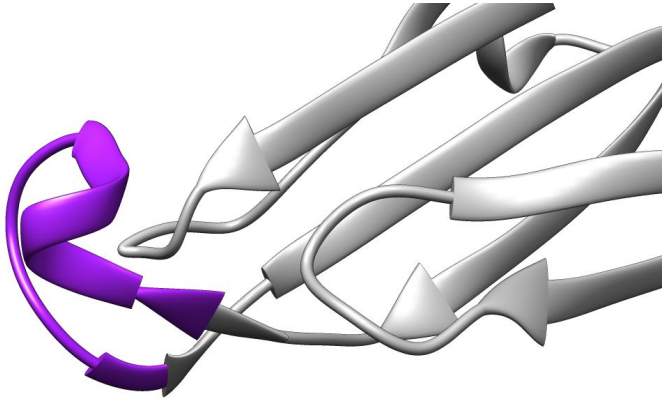
```
Zika_virus_1/1-504                  LYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA 250
Zika_virus_2/1-498                  LYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA 244
Japanese_encephalitis_virus_1/1-500 FYVMTVGSKSFLVHREWFHDLALPWTPPSST---AWRNRELLMEFEEAHA 247
Japanese_encephalitis_virus_2/1-499 FYVMTVGSKSFLVHREWFHDLALPWTSPSST---AWRNRELLMEFEEAHA 246
West_Nile_virus_1/1-501             YYVMTVGTKTFLVHREWFMDLNLPWSSAGST---VWRNRETLMEFEEPHA 247
West_Nile_virus_2/1-501             YYVMSVGAKSFLVHREWFMDLNLPWSSAGST---TWRNRETLMEFEEPHA 247
Dengue_virus_4_1/1-495              MILMKMKKKTWLVHKQWFLDLPLPWTTGADTSEVHWNYKERMVTFKVPHA 245
Dengue_virus_4_2/1-495              MILMKMNKKTWLVHKQWFLDLPLPWTAGADTSEVHWNNKERMVTFKVPHA 245
Dengue_virus_1_1/1-495              MVLLTMKEKSWLVHKQWFLDLPLPWTSGASTSQETWNRQDLLVTFKTAHA 245
Dengue_virus_1_2/1-495              MVLLTMKEKSWLVHKQWFLDLPLPWTSGASTSQETWNRQDLLVTFKTAHA 245
Dengue_virus_3_1/1-493              MILLTMKNKAWMVHRQWFFDLPLPWTSGATTETPTWNKKELLVTFKNAHA 243
Dengue_virus_3_2/1-493              MILLTMKNKAWMVHRQWFFDLPLPWTSGATTETPTWNRKELLVTFKNAHA 243
Dengue_virus_2_1/1-495              MVLLQMENKAWLVHRQWFLDLPLPWLPGADKQESNWIQKETLVTFKNPHA 245
Dengue_virus_2_2/1-495              MVLLQMEDKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTFKNPHA 245
Yellow_fever_virus_1/1-493          SYIAEMEKESWIVDKQWAQDLTLPWQSGSGG---VWREMHHLVEFEPPHA 239
Yellow_fever_virus_2/1-493          SYIAEMEKESWIVDKQWAQDLTLPWQSGSGG---VWREMHHLVEFEPPHA 239
                                         :  : ::*.::*  *: *** . .      *   . :: *: .**
```

E PROTEIN: CONSERVED LOOPS



Fusion Loop

Virus mediated membrane fusion



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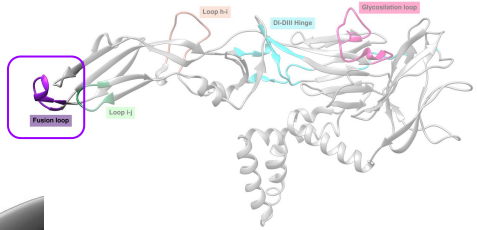
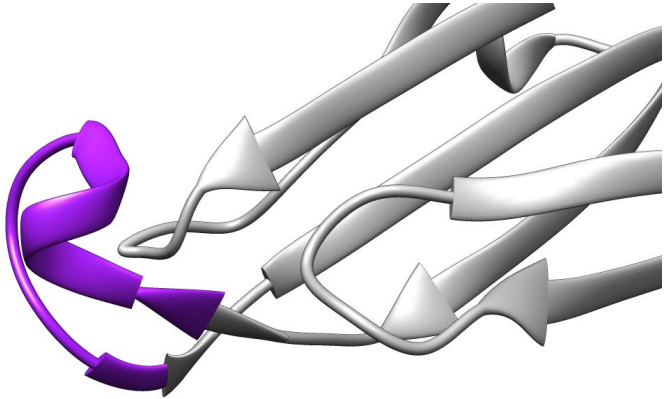
Zika_virus_1/1-504                  SNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCA
Zika_virus_2/1-498                  SNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCA
Japanese_encephalitis_virus_1/1-500 SQLAEVRSYCYHASVTDISTVARCPMTGEAHNEKRADSSYVCKQGFTDRGWGNGCGLFGKGSIDTCA
Japanese_encephalitis_virus_2/1-499 SQLAEVRSYCYHASVTDISTVARCPTTGEAHNEKRADSSYVCKQGFTDRGWGNGCGLFGKGSIDTCA
West_Nile_virus_1/1-501             ANLAEVRSYCYLAAVSDLSTKAACPTMGEAHNDKRADPAFVCRQGVVDRGWGNGCGLFGKGSIDTCA
West_Nile_virus_2/1-501             ANLADVRSYCYLASVSDLSTRAACPTMGEAHNEKRADPAFVCKQGVVDRGWGNGCGLFGKGSIDTCA
Dengue_virus_4_1/1-495              KEVALLRTYCIEALISNITTATRCPTQGEPYLKEEQDQQYICRRDVVDRGWGNGCGLFGKGGVVTCA
Dengue_virus_4_2/1-495              KEVALLRTYCIEASISNITTATRCPTQGEPYLKEEQDQQYICRRDVVDRGWGNGCGLFGKGGVVTCA
Dengue_virus_1_1/1-495              TNPAVLRKLCIEAKISNTTTDSRCPTQGEATLVEEQDANFVCRRTFVDRGWGNGCGLFGKGSLITCA
Dengue_virus_1_2/1-495              TNPAVLRKLCIEAKISNTTTDSRCPTQGEATLVEEQDANFVCRRTFVDRGWGNGCGLFGKGSLLTCA
Dengue_virus_3_1/1-493              TQLATLRKLCIEGKITNVTTDSRCPTQGEAILPEEQDQNYVCKHTYVDRGWGNGCGLFGKGSLVTCA
Dengue_virus_3_2/1-493              TQLATLRKLCIEGKITNITTDSRCPTQGEAILPEEQDQNYVCKHTYVDRGWGNGCGLFGKGSLVTCA
Dengue_virus_2_1/1-495              KQPATLRKYCIEAKLTNTTTESRCPTQGEPSLKEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCA
Dengue_virus_2_2/1-495              KQPATLRKYCIEAKLTNTTTESRCPTQGEPSLNEEQDKRFICKHSMVDRGWGNGCGLFGKGGIVTCA
Yellow_fever_virus_1/1-493          DGPAEARKVCYSAVLTNVKINDKCPSTGEAHLEEENEGDNACKRTYSDRGWGNGCGLFGKGSIVACA
Yellow_fever_virus_2/1-493          DGPAEARKVCYSAVLTNVKINDKCPSTGEAHLEEENEGDNACKRTYSDRGWGNGCGLFGKGSIVACA
                                       *  *. *  . :::      **  **.   :. :    *::   ***********. : :**
```

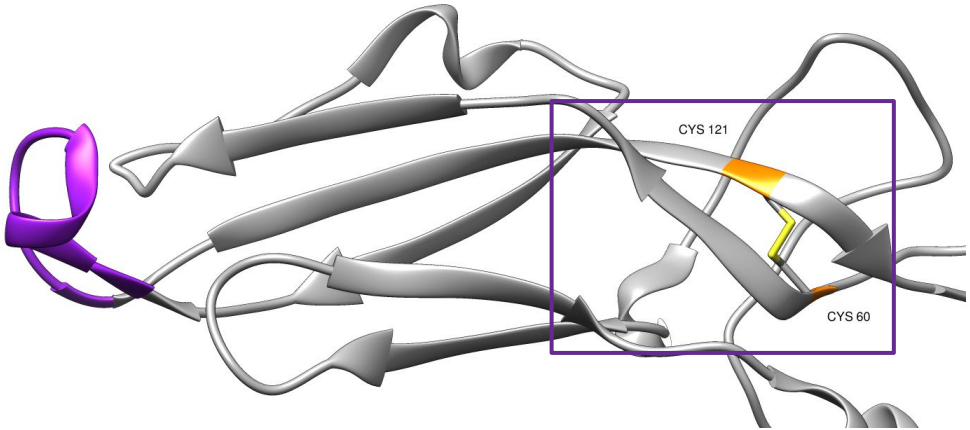
E PROTEIN: CONSERVED LOOPS



Fusion Loop

Virus mediated membrane fusion





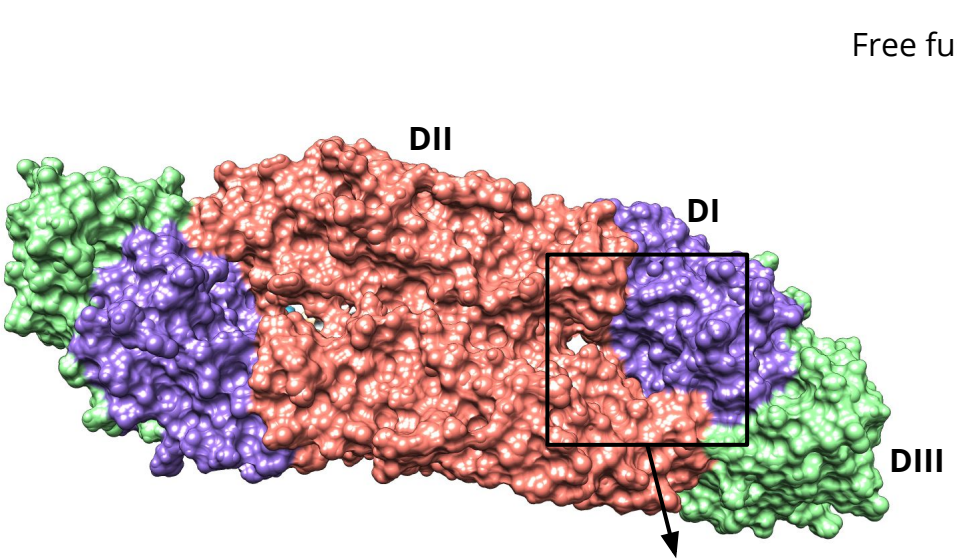
HYDROPHOBIC POCKET

VIRUS MEDIATED MEMBRANE FUSION AND DRUG TARGET

HOMODIMER
Pre-fusion conformation

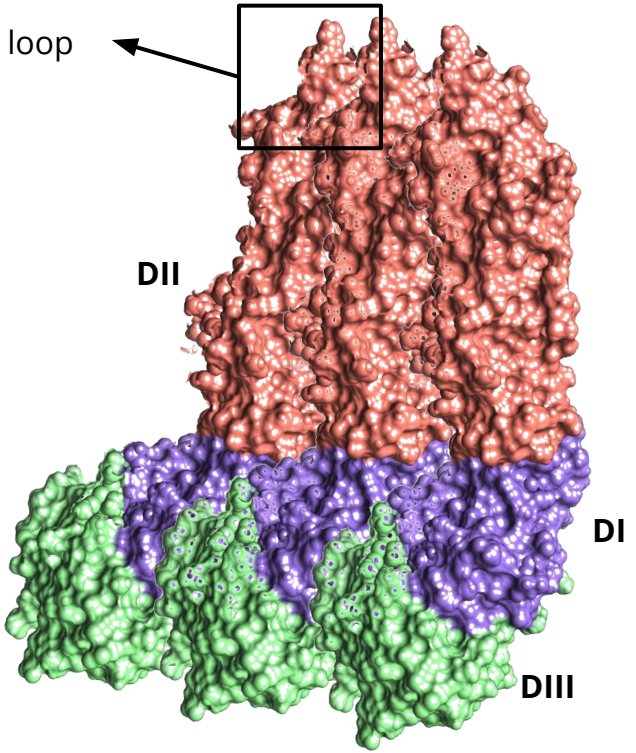
low pH

FUSOGENIC TRIMER
Fusion conformation



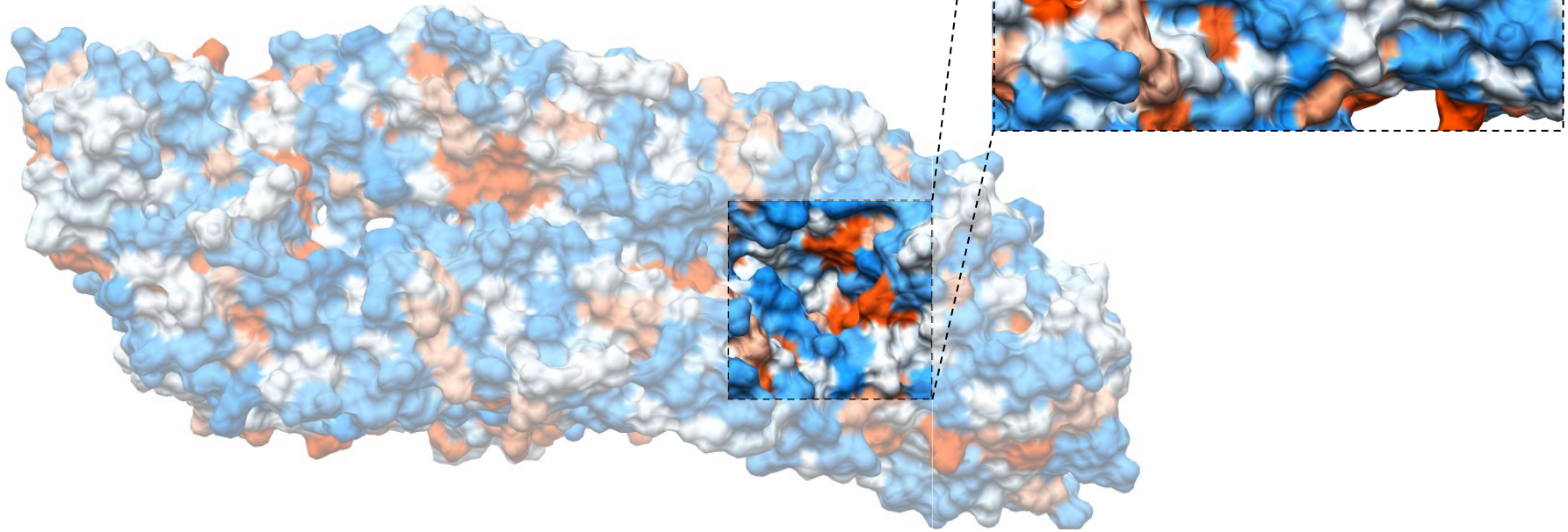
Fusion loop buried by the hydrophobic pocket

Free fusion loop



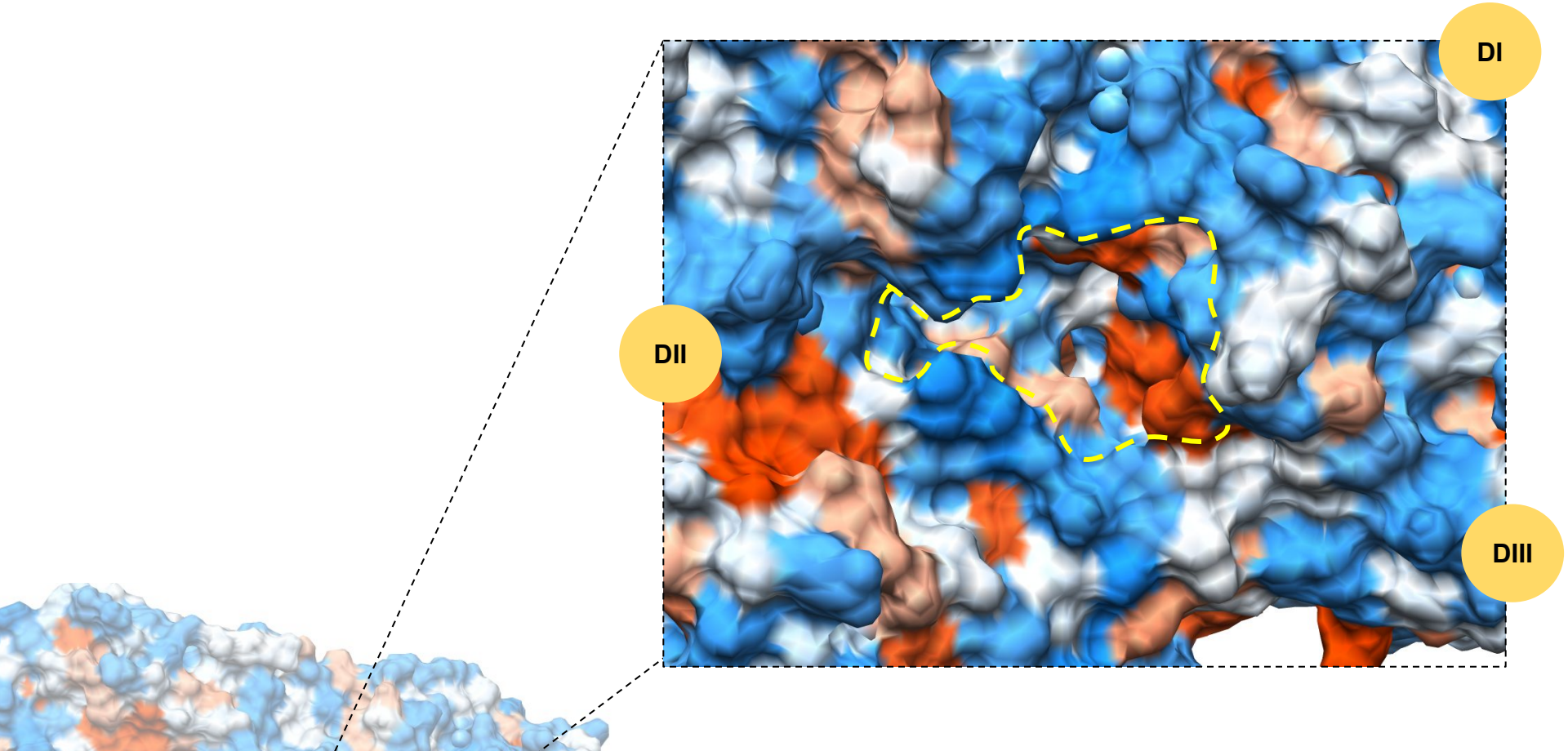
HYDROPHOBIC POCKET

VIRUS MEDIATED MEMBRANE FUSION
AND DRUG TARGET



HYDROPHOBIC POCKET

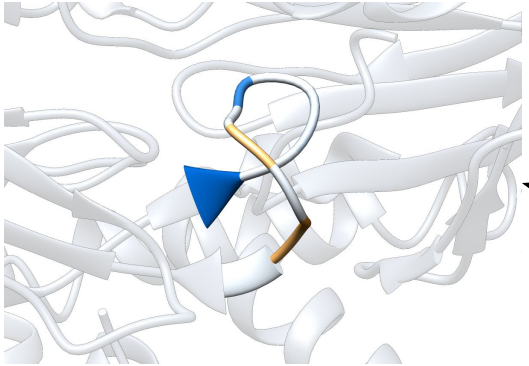
VIRUS MEDIATED MEMBRANE FUSION AND DRUG TARGET

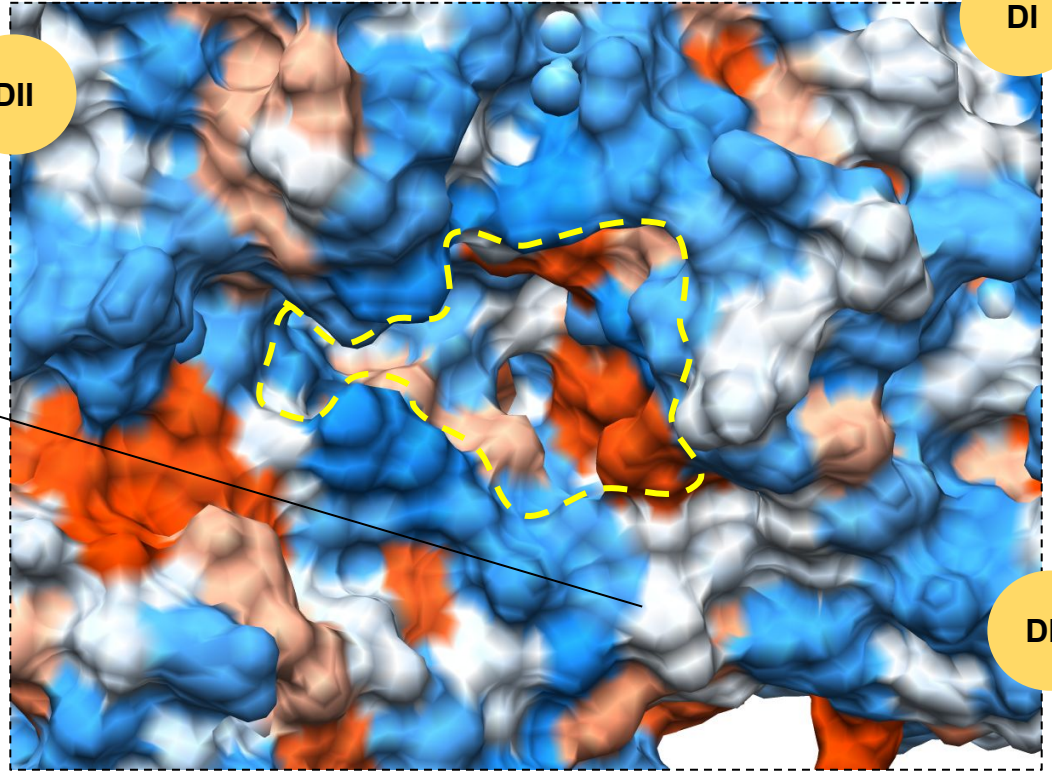


HYDROPHOBIC POCKET

VIRUS MEDIATED MEMBRANE FUSION AND DRUG TARGET

fusion-loop

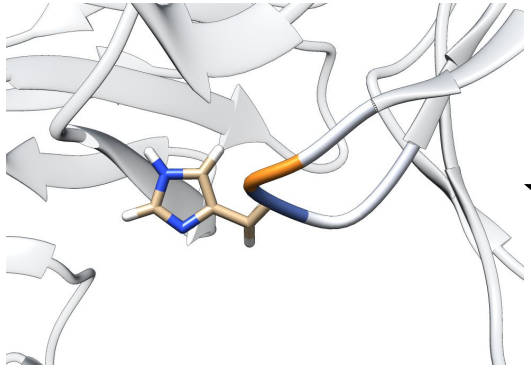


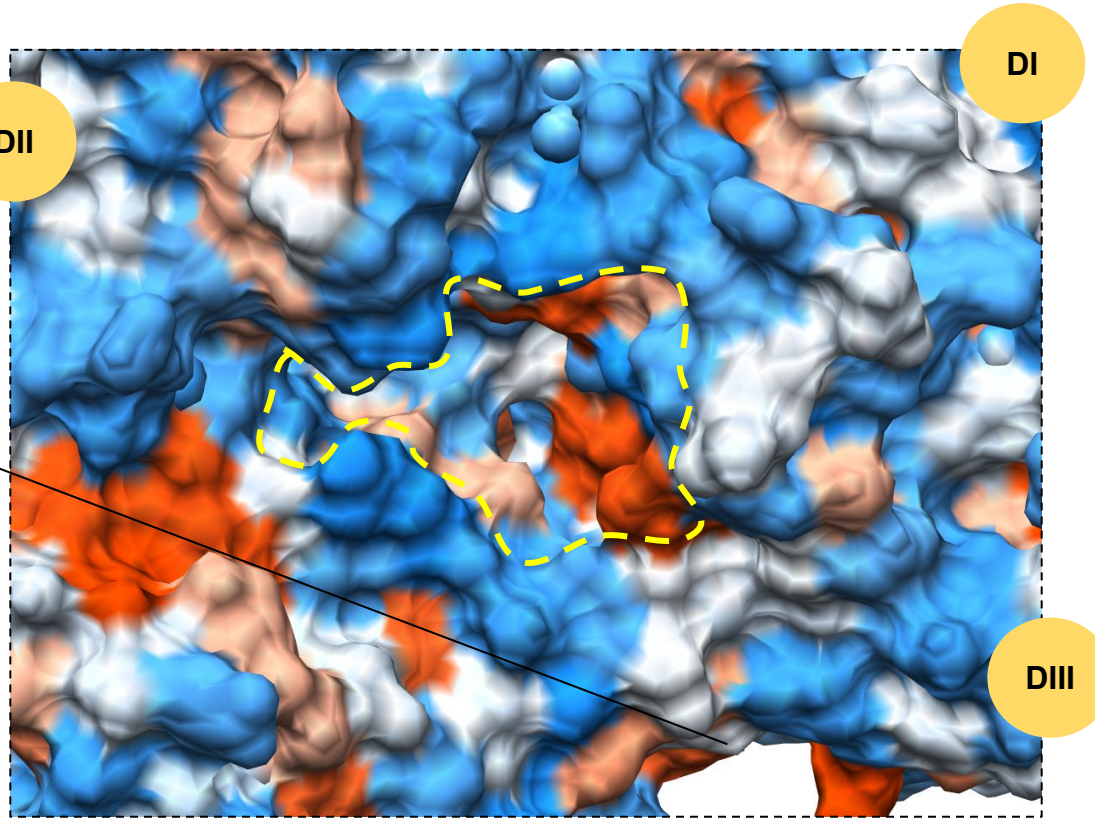


HYDROPHOBIC POCKET

VIRUS MEDIATED MEMBRANE FUSION AND DRUG TARGET

HIS 323

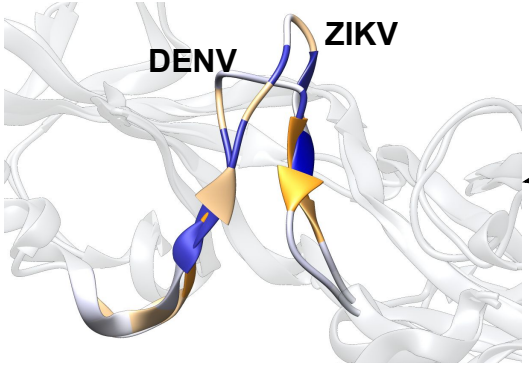


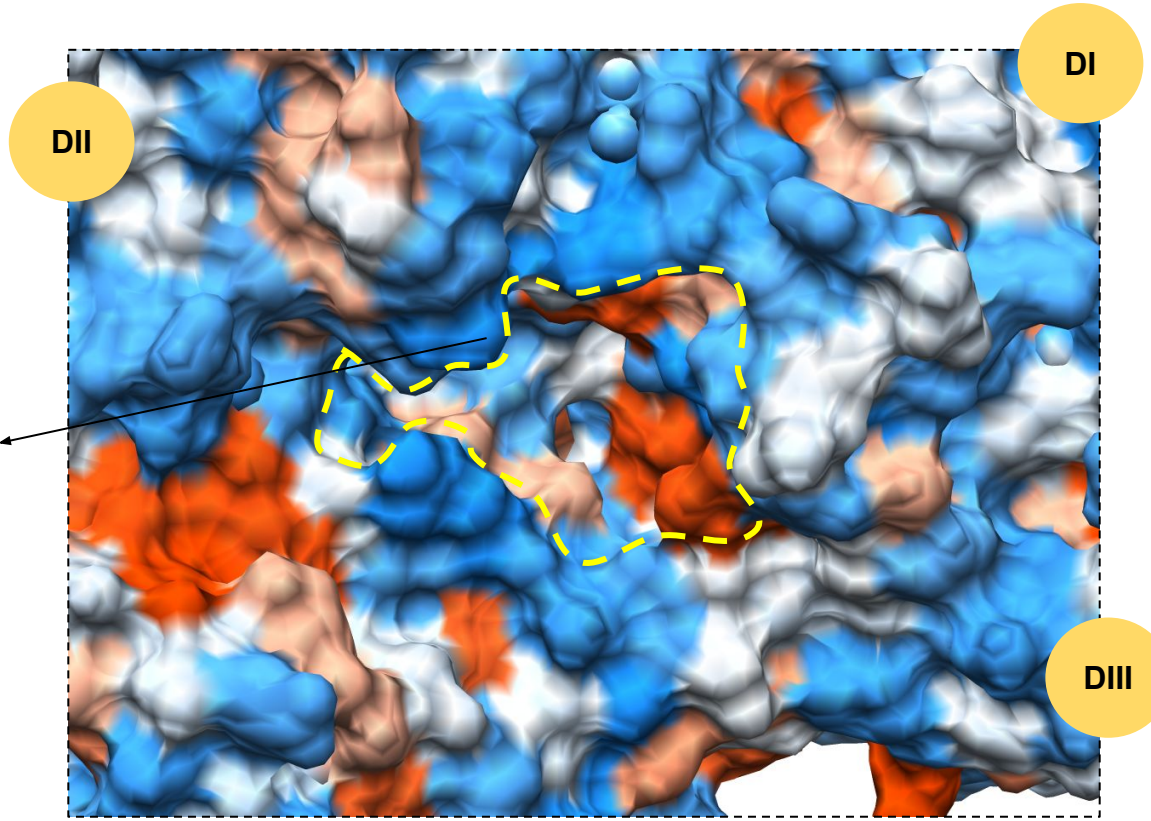


HYDROPHOBIC POCKET

VIRUS MEDIATED MEMBRANE FUSION AND DRUG TARGET

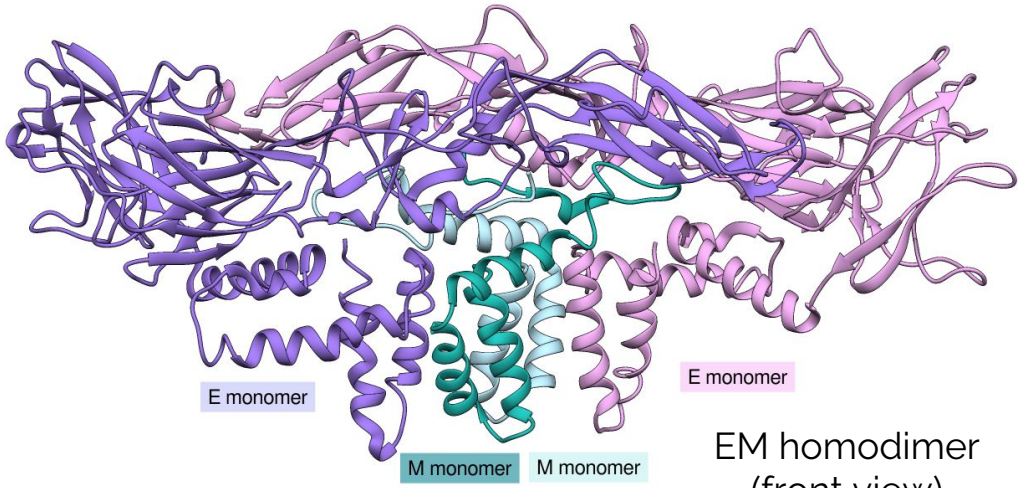
(k-l Loop)
beta-hairpin





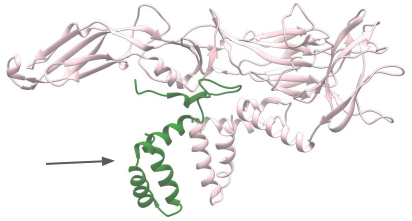
Envelope elements

M protein



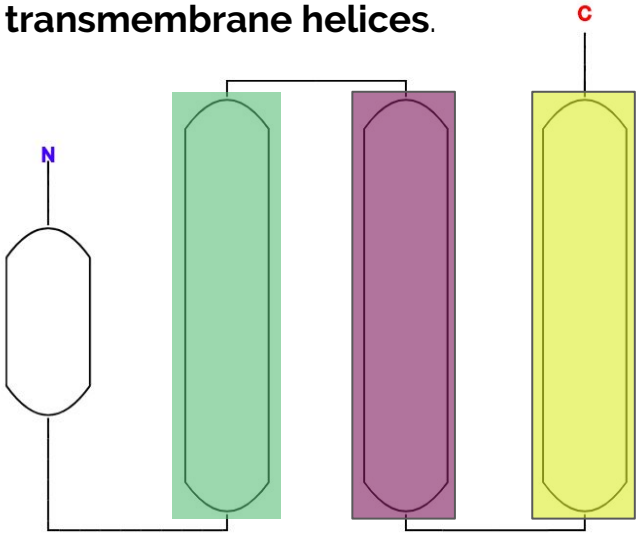
EM homodimer
(front view)

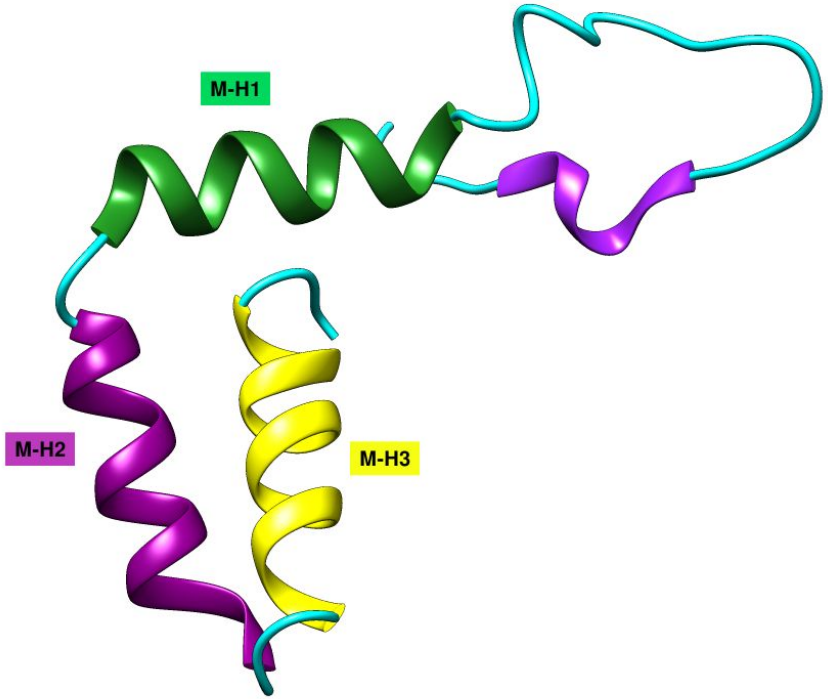
M PROTEIN: TOPOLOGICAL DIAGRAM



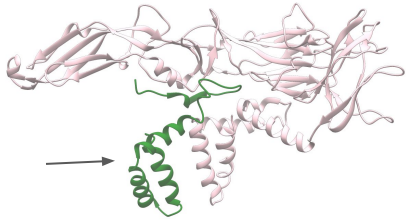
Has three helices: M-H1, M-H2, and M-H3.

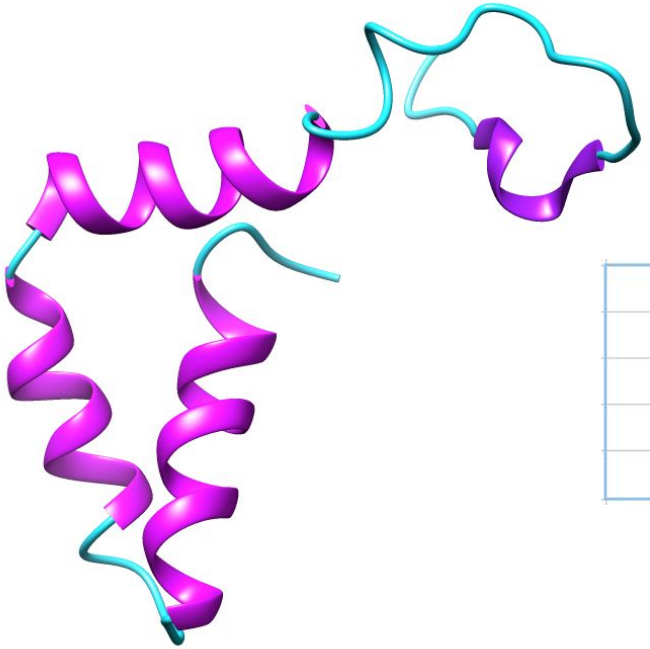
The helices **M-H2 and M-H3 are transmembrane helices**.





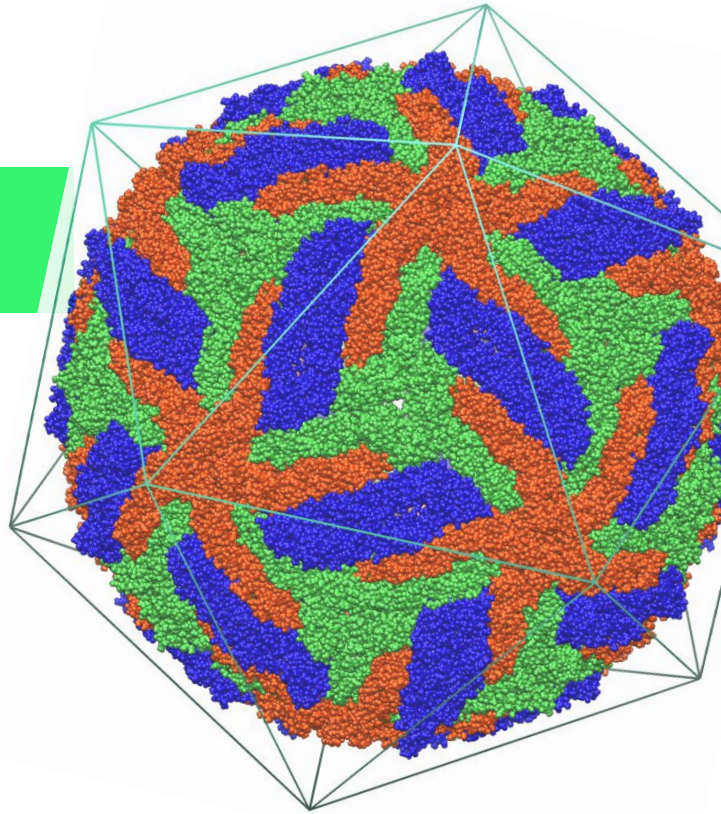
M PROTEIN



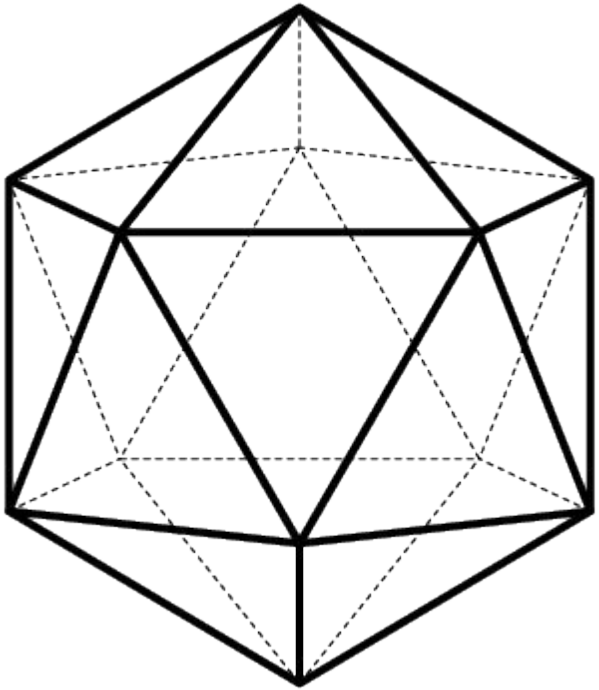


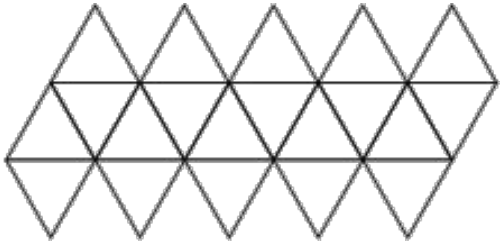
Level	
C	Mainly Alpha
A	Orthogonal Bundle
T	Helicase, Ruva Protein; domain 3
H	Flavivirus envelope glycoprotein M-like

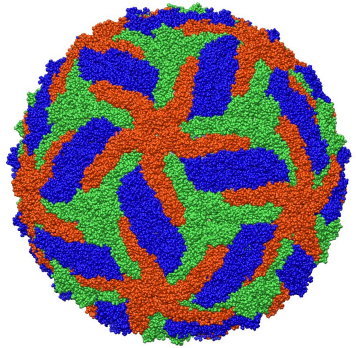
Envelope symmetry



ENVELOPE SYMMETRY
ICOSAHEDRAL ASSEMBLY



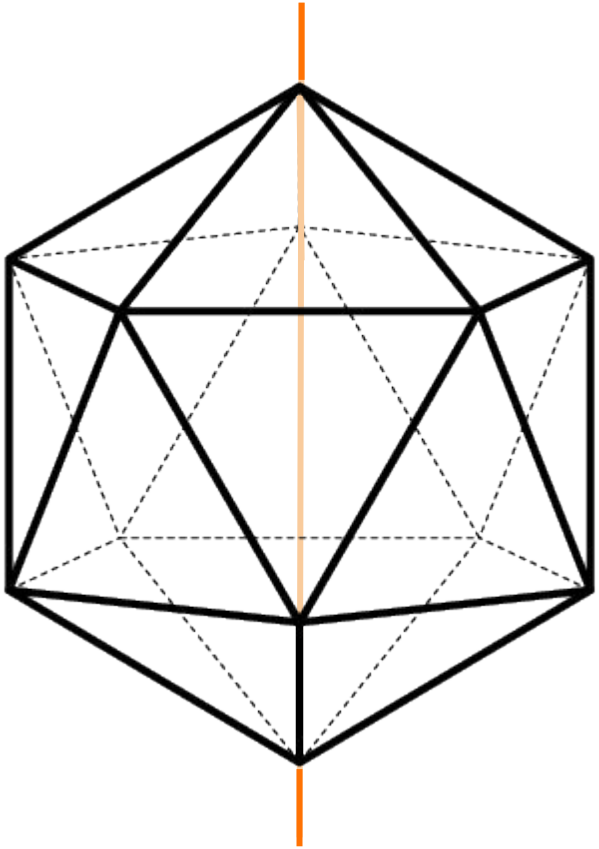


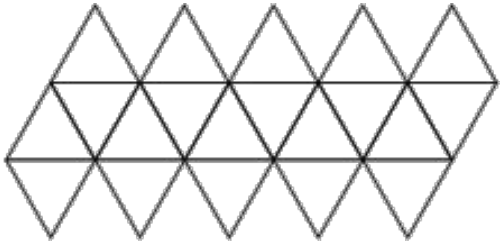


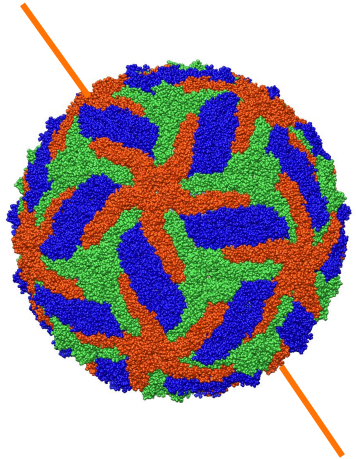
Symmetry axes:

- **Six 5-fold axes through the 12 vertices**
- **Ten 3-fold axes through the 20 triangular faces**
- **Fifteen 2-fold axes through the 30 edges**

ENVELOPE SYMMETRY
ICOSAHEDRAL ASSEMBLY







Symmetry axes:

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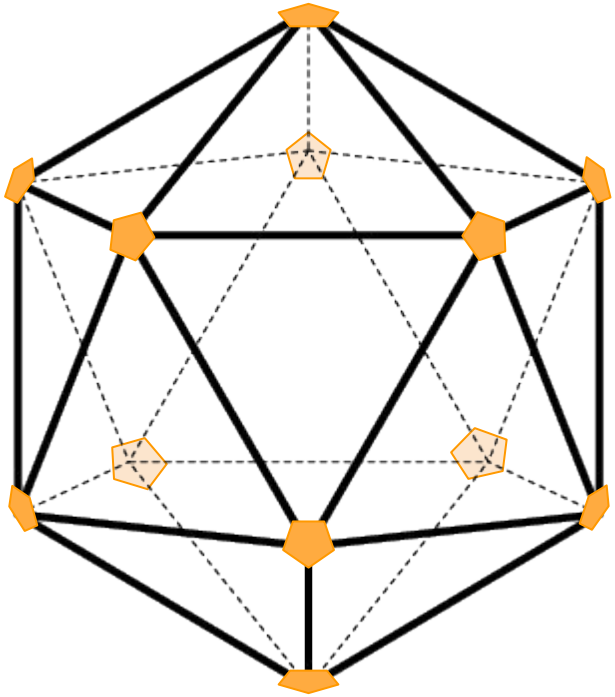
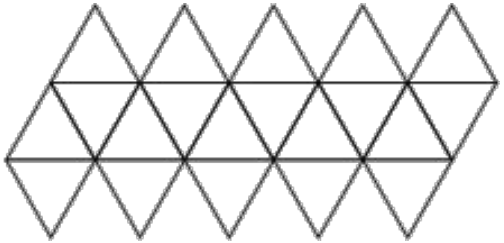
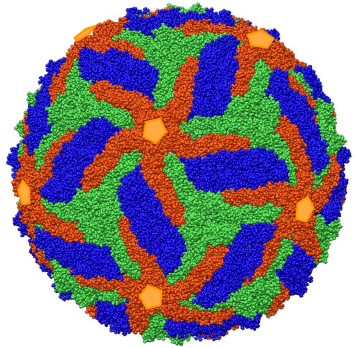
- **Ten 3-fold axes through the 20 triangular faces**
- **Fifteen 2-fold axes through the 30 edges**

ENVELOPE SYMMETRY
ICOSAHEDRAL ASSEMBLY





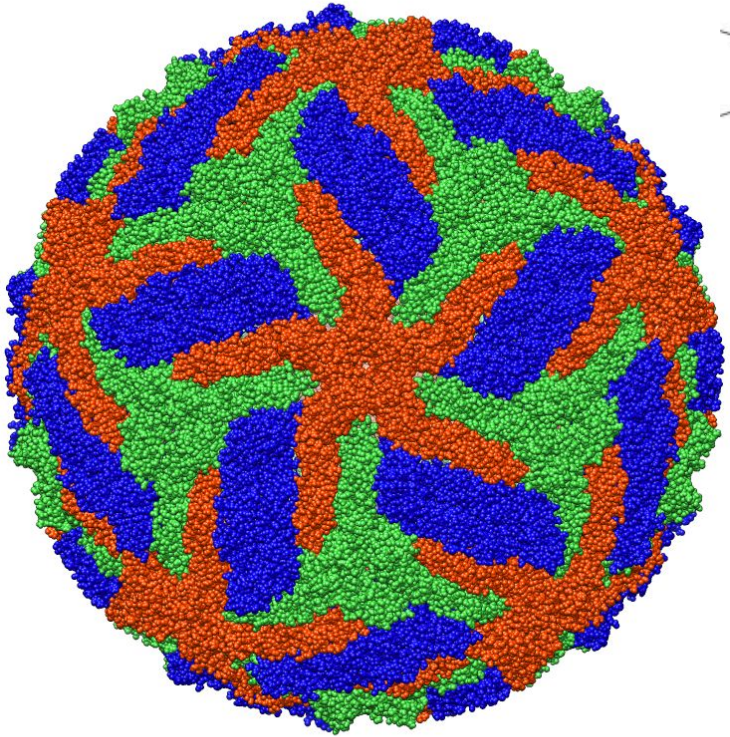


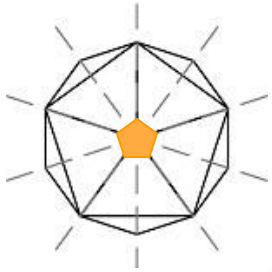
Symmetry axes:

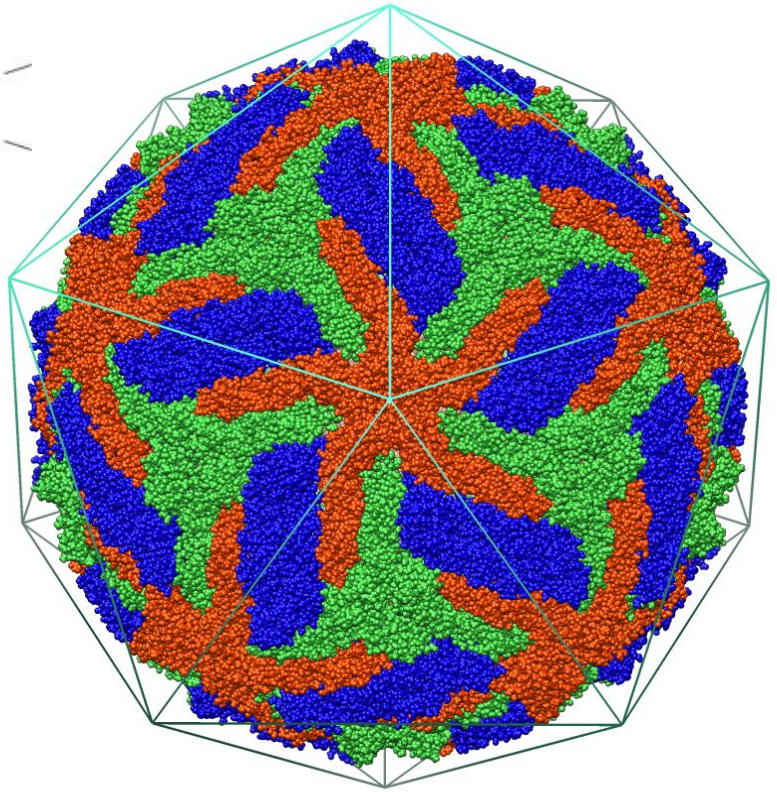
- **Six 5-fold axes through the 12 vertices**
- **Ten 3-fold axes through the 20 triangular faces**
- **Fifteen 2-fold axes through the 30 edges**

ENVELOPE SYMMETRY

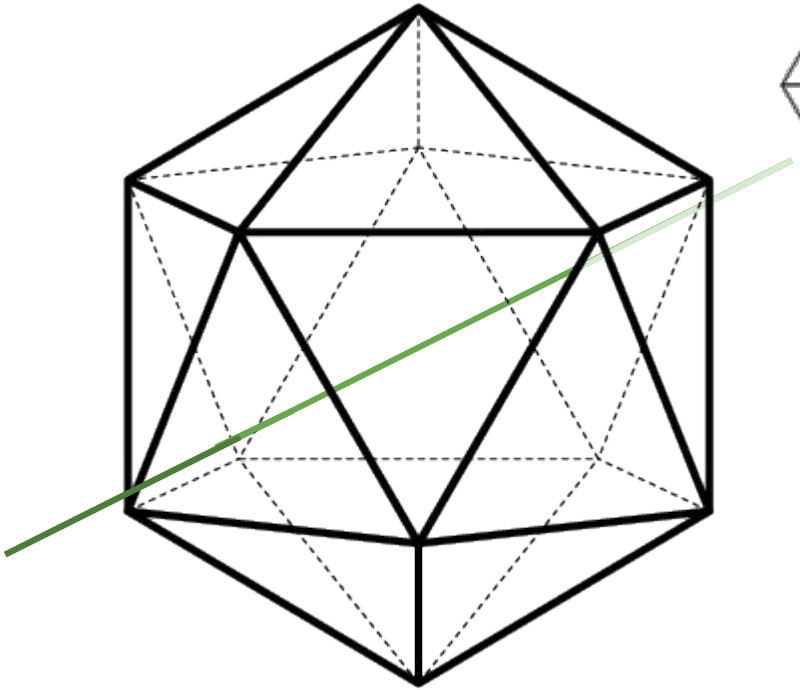
5-FOLD AXIS

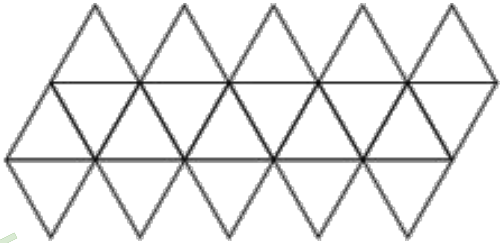


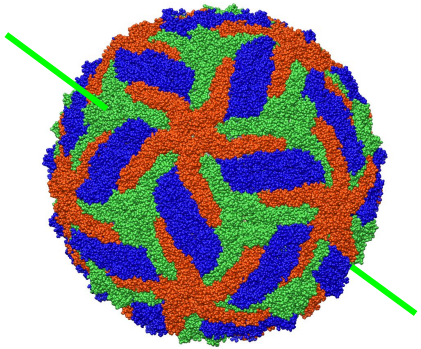




ENVELOPE SYMMETRY
ICOSAHEDRAL ASSEMBLY







Symmetry axes:

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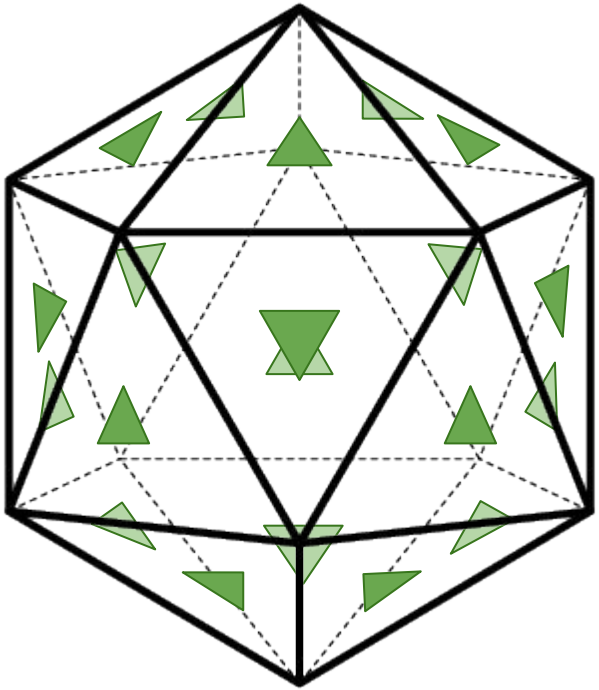
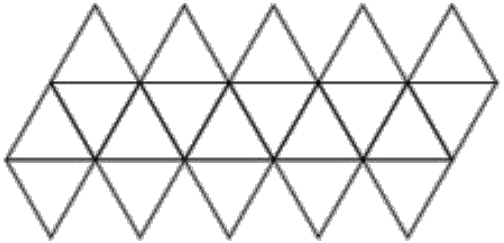
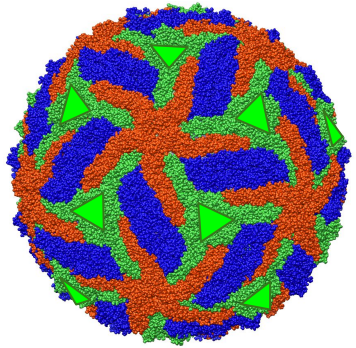
- **Ten 3-fold axes through the 20 triangular faces**
- **Fifteen 2-fold axes through the 30 edges**

ENVELOPE SYMMETRY
ICOSAHEDRAL ASSEMBLY





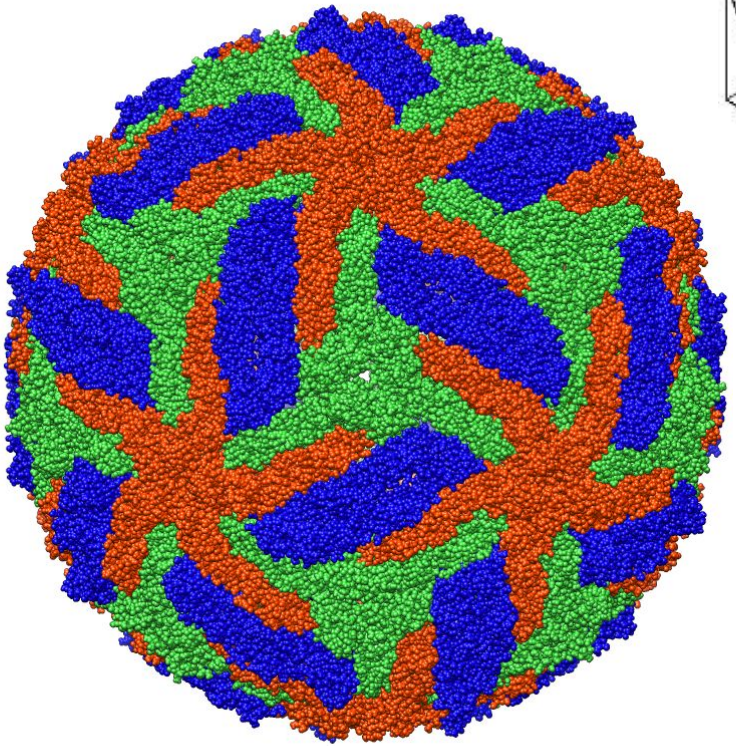


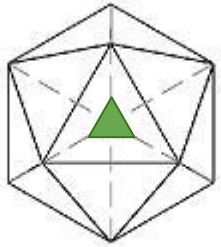
Symmetry axes:

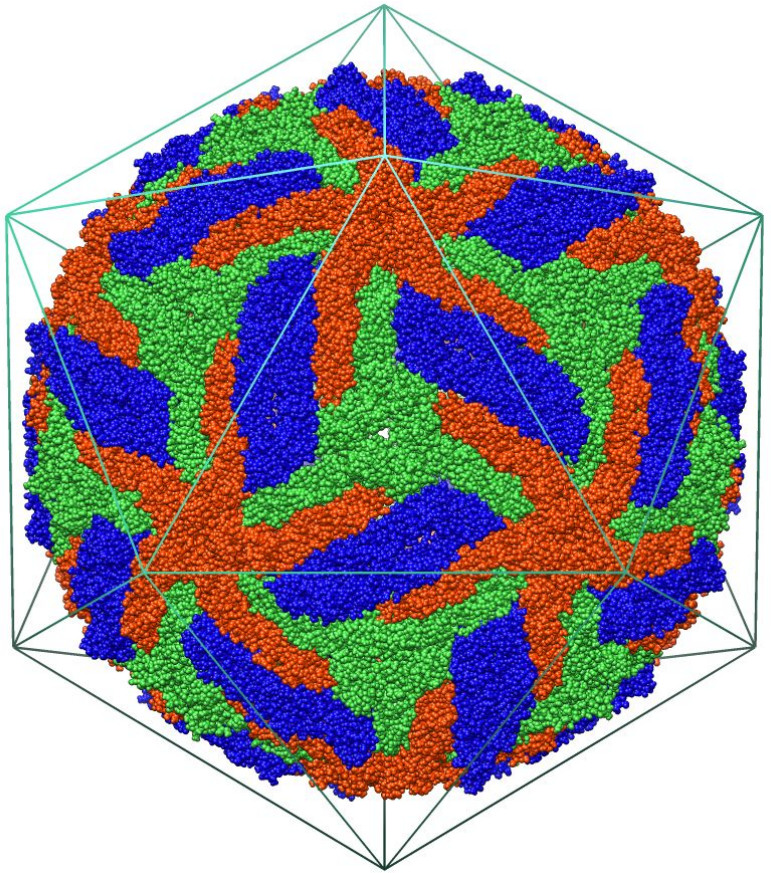
- **Six 5-fold axes through the 12 vertices**
- **Ten 3-fold axes through the 20 triangular faces**
- **Fifteen 2-fold axes through the 30 edges**

ENVELOPE SYMMETRY

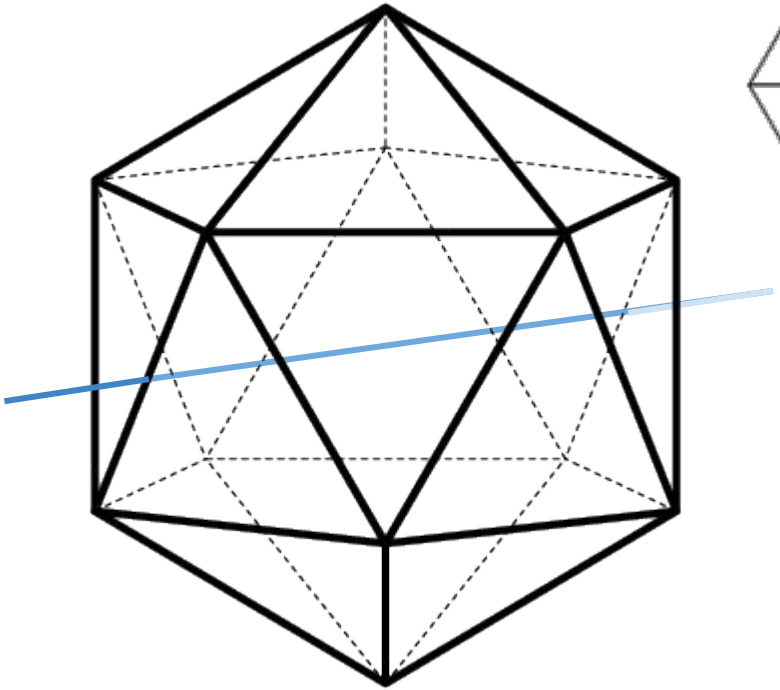
3-FOLD AXIS

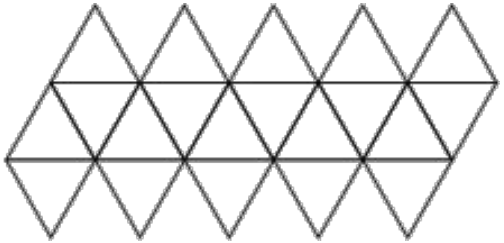


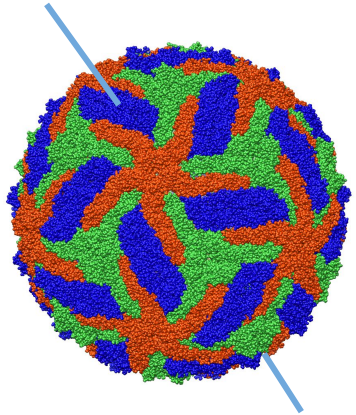




ENVELOPE SYMMETRY
ICOSAHEDRAL ASSEMBLY



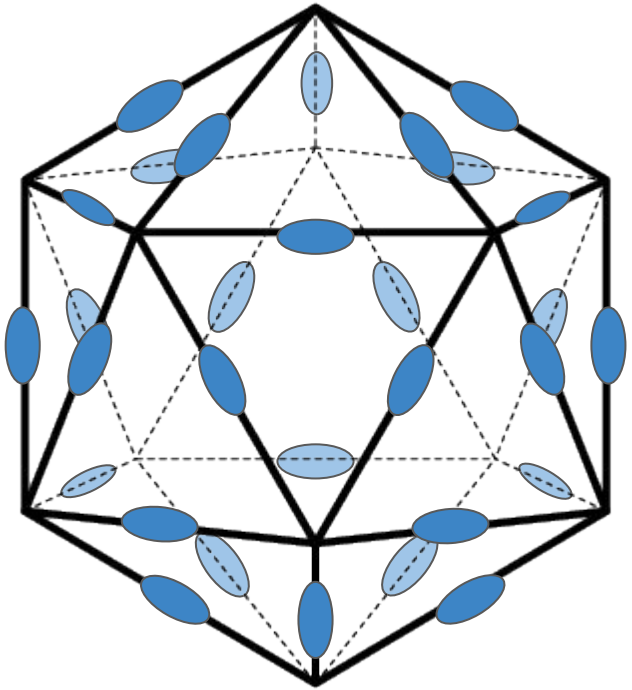


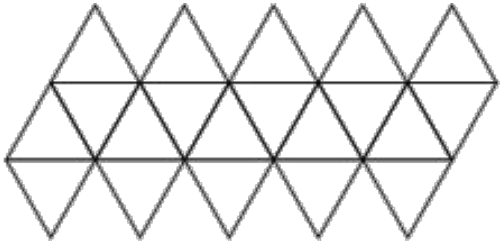


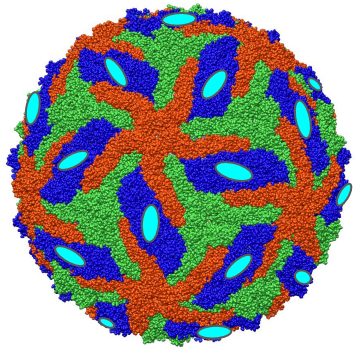
Symmetry axes:

- Six 5-fold axes through the 12 vertices
- Ten 3-fold axes through the 20 triangular faces
- Fifteen 2-fold axes through the 30 edges

ENVELOPE SYMMETRY
ICOSAHEDRAL ASSEMBLY





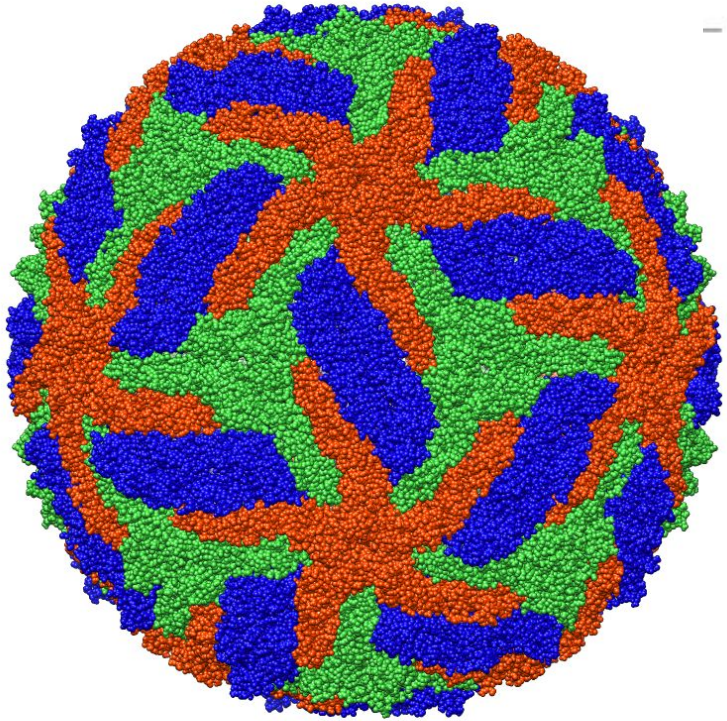


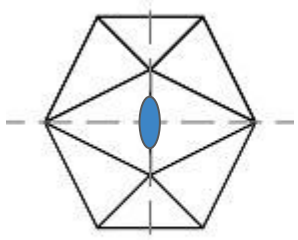
Symmetry axes:

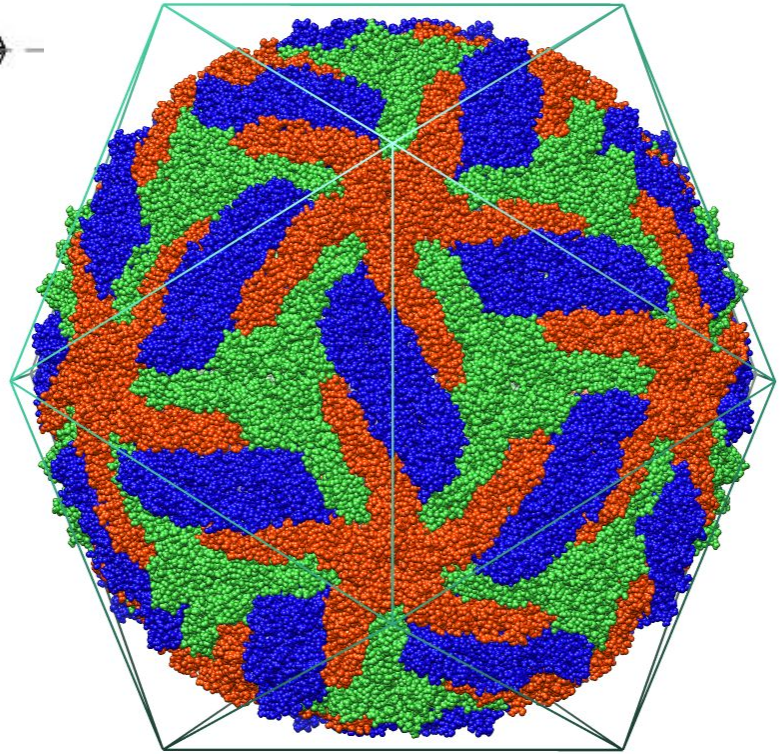
- Six 5-fold axes through the 12 vertices
- Ten 3-fold axes through the 20 triangular faces
- Fifteen 2-fold axes through the 30 edges

ENVELOPE SYMMETRY

2-FOLD AXIS

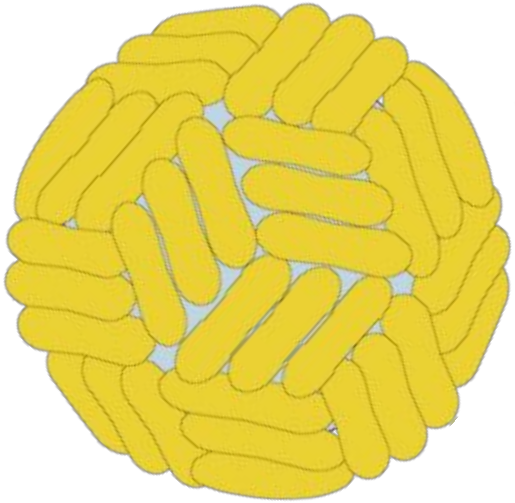


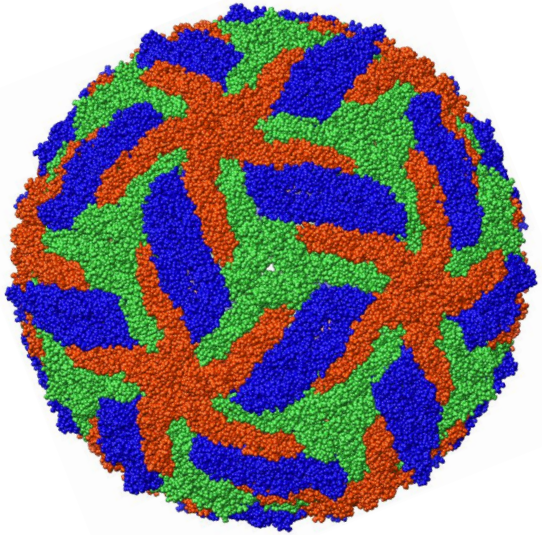




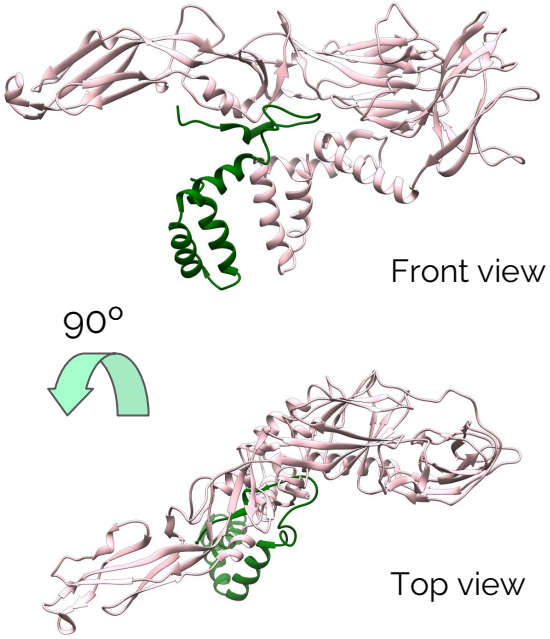
ENVELOPE SYMMETRY
GLYCOPROTEIN SHELL

Herringbone pattern of 180 E proteins (90 antiparallel dimers of E protein)



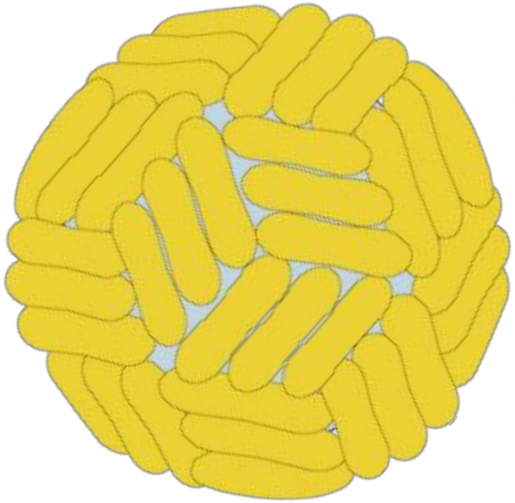


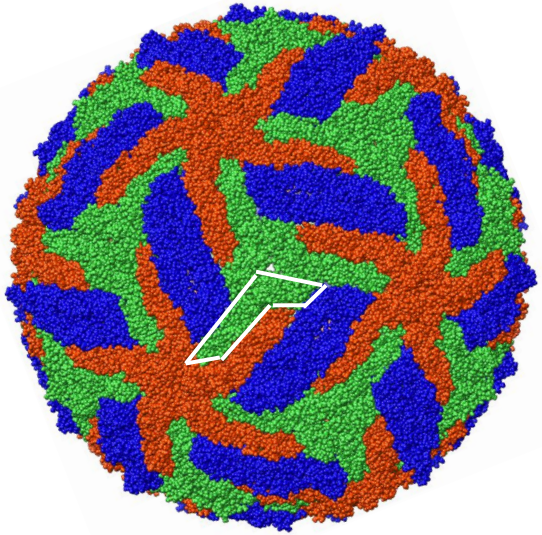
Unit structure: E-M heterodimer



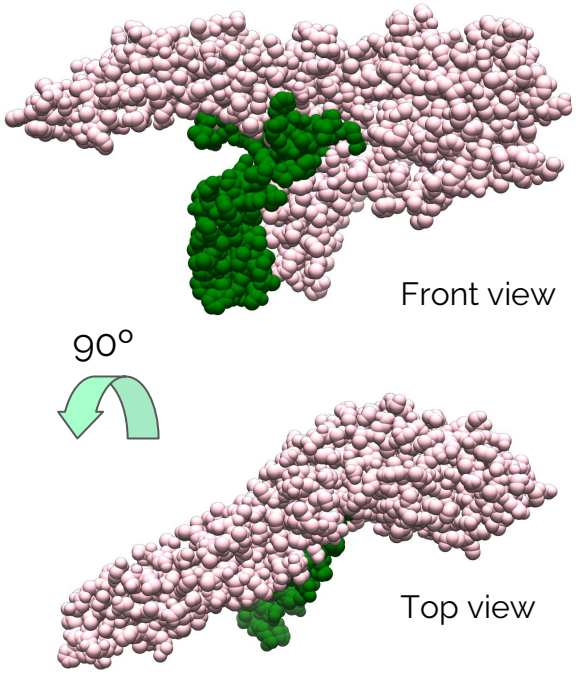
ENVELOPE SYMMETRY
GLYCOPROTEIN SHELL

Herringbone pattern of 180 E proteins (90 antiparallel dimers of E protein)





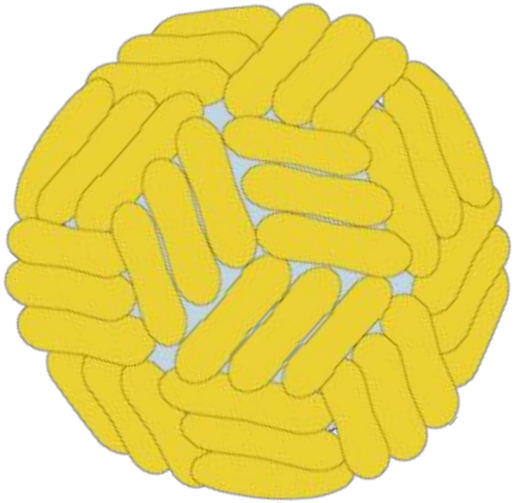
Unit structure: E-M heterodimer

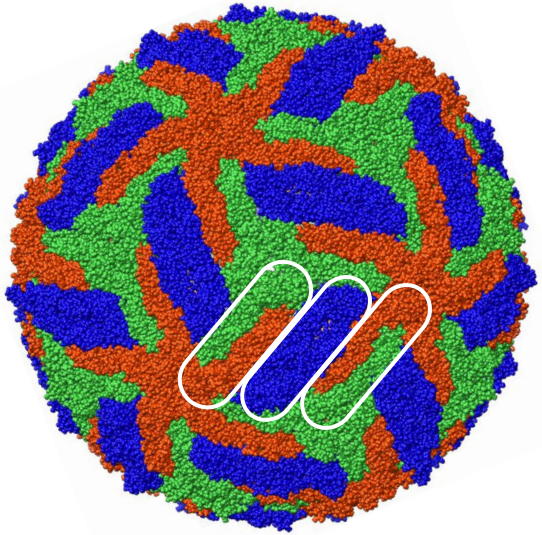


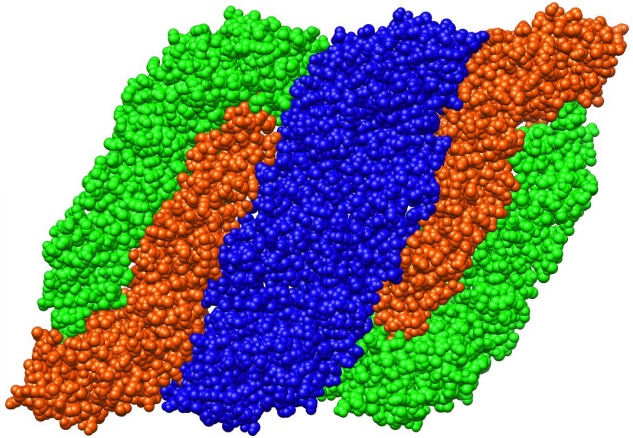
ENVELOPE SYMMETRY
GLYCOPROTEIN SHELL

Herringbone pattern of 180 E proteins (90 antiparallel dimers of E protein)

Three E dimers form a raft

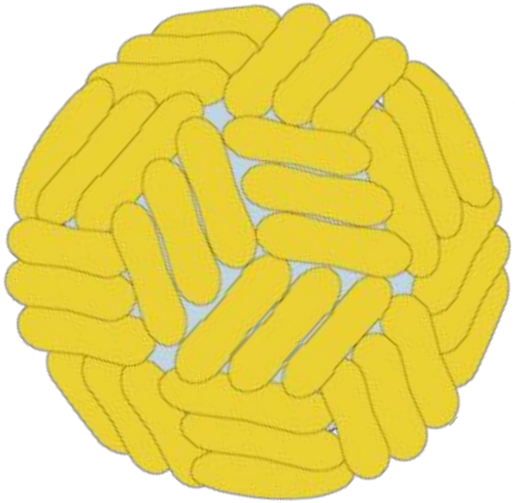


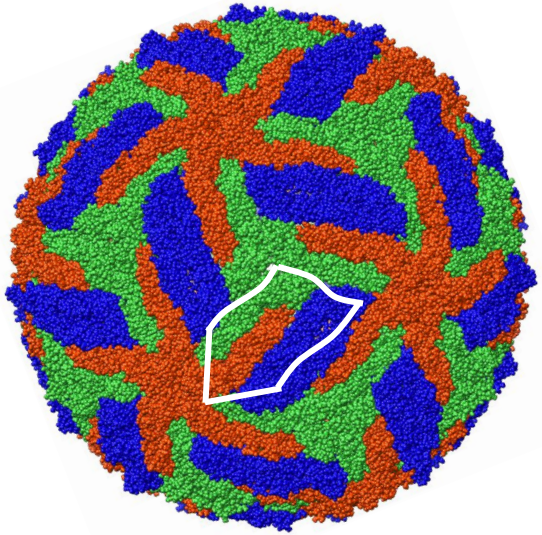




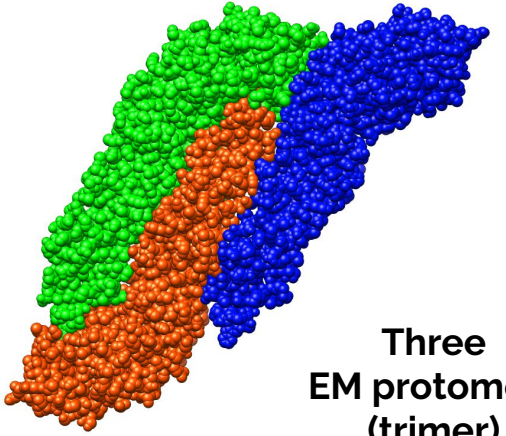
ENVELOPE SYMMETRY
ASYMMETRIC UNIT

Herringbone pattern of 180 E proteins (90 antiparallel dimers of E protein)



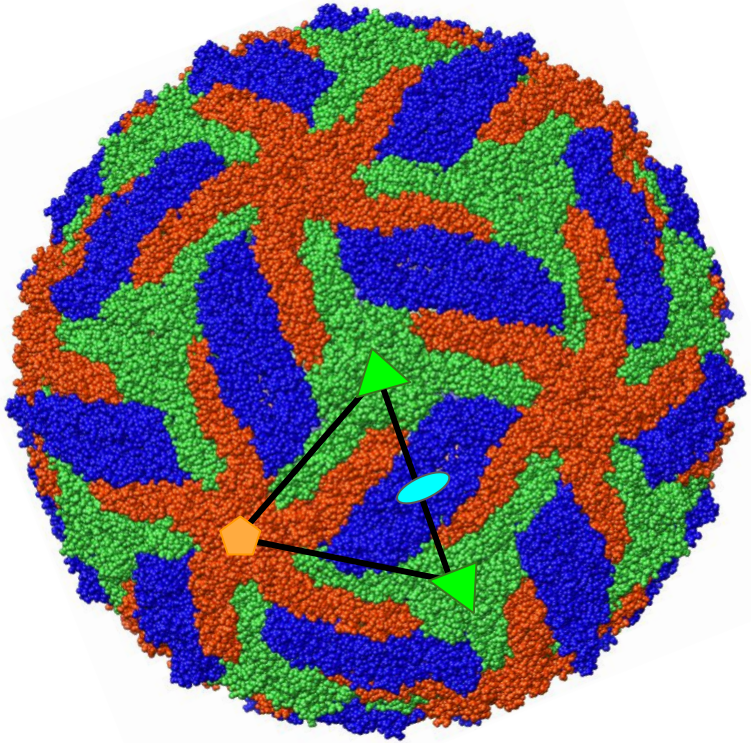


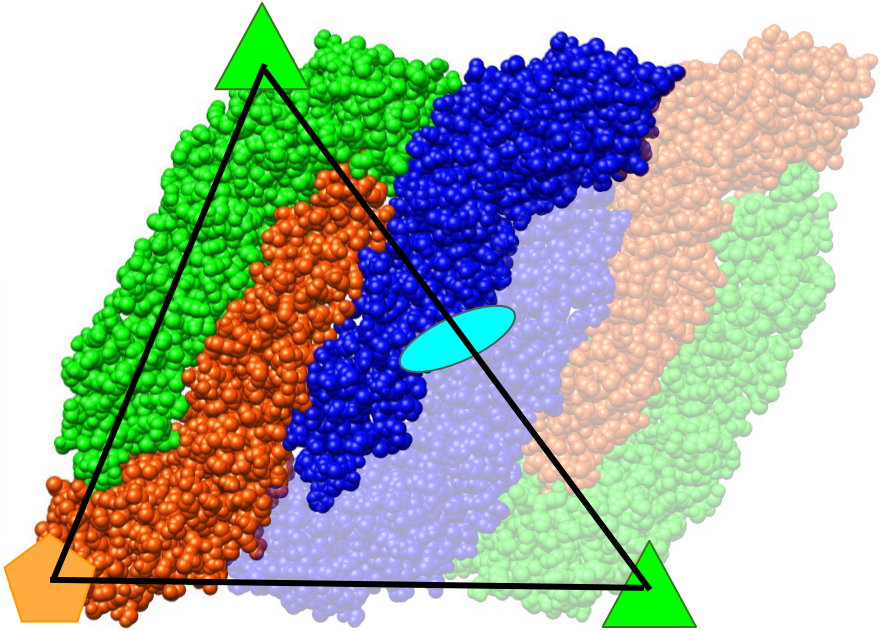
60 asymmetric units
(T=3-like organization of surface EM heterodimers)



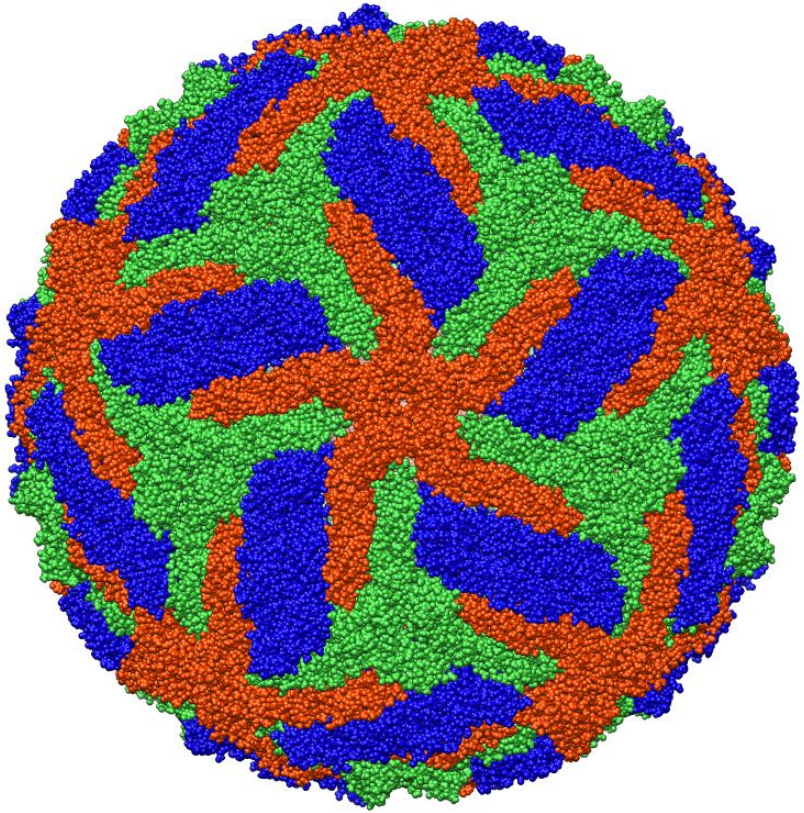
Three EM protomers (trimer)

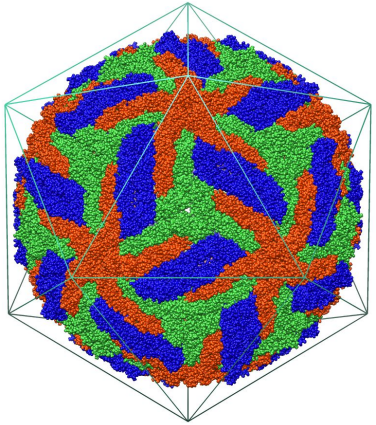
ENVELOPE SYMMETRY
ASYMMETRIC UNIT



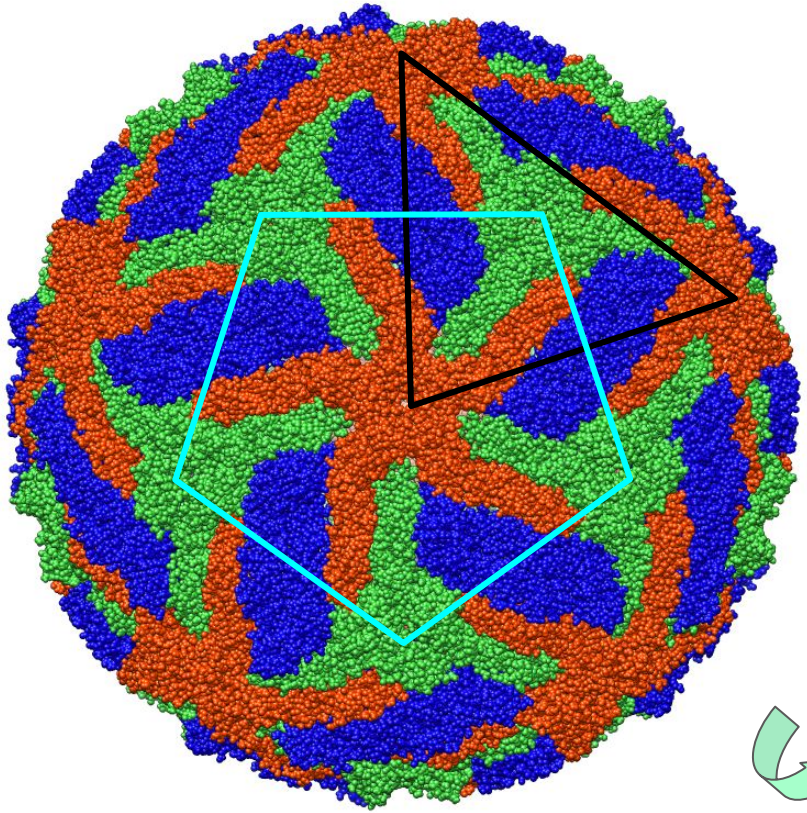


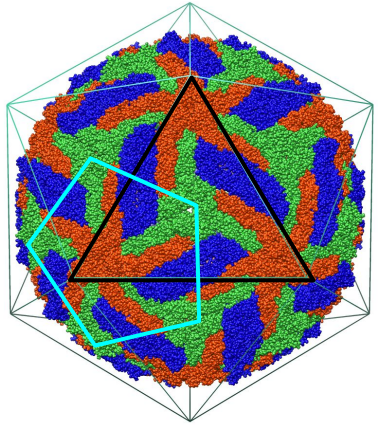
ENVELOPE SYMMETRY
ASYMMETRIC UNIT



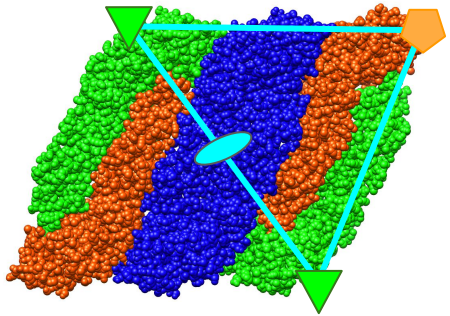


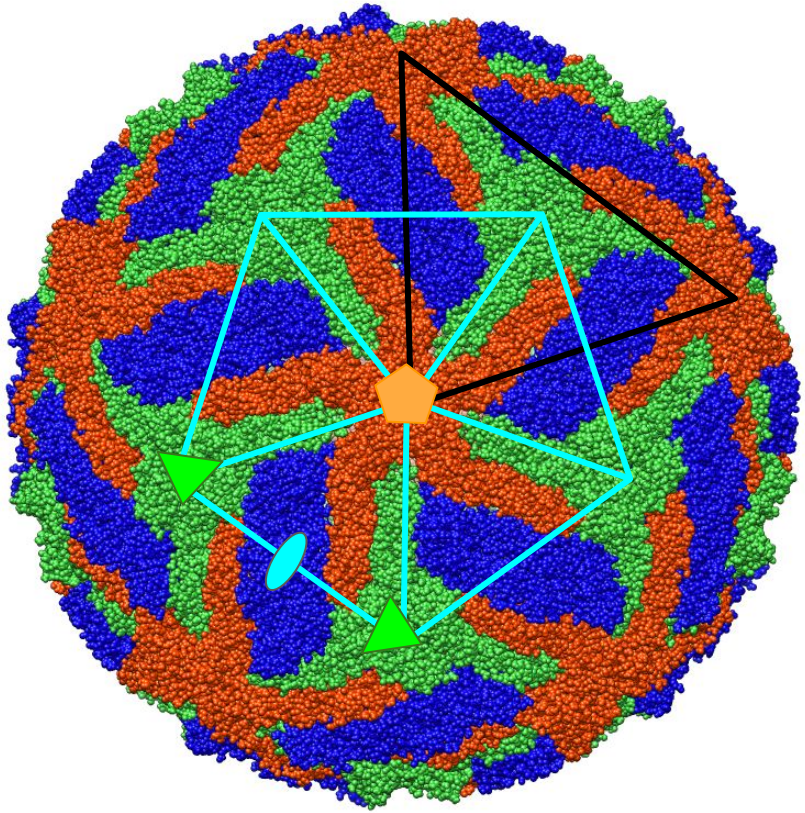
ENVELOPE SYMMETRY
ASYMMETRIC UNIT

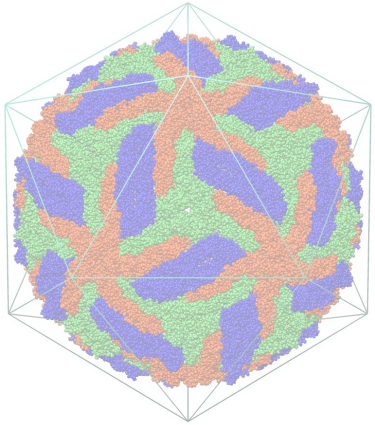




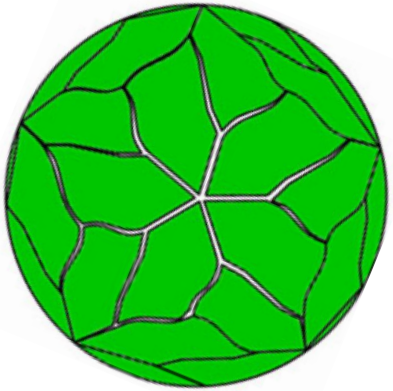
ENVELOPE SYMMETRY
ASYMMETRIC UNIT

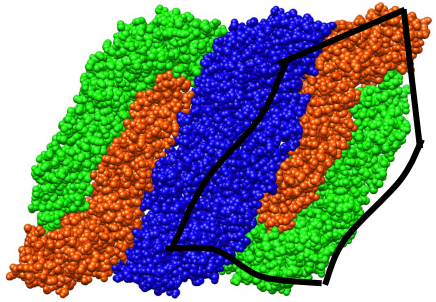


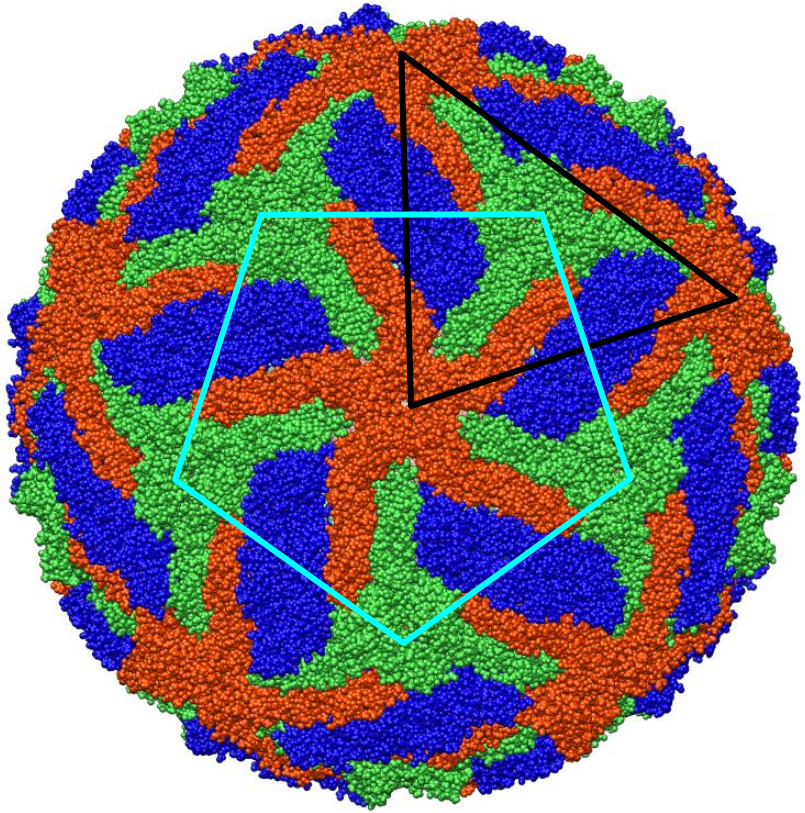


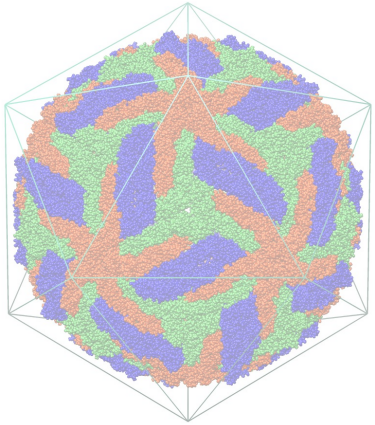


ENVELOPE SYMMETRY
ASYMMETRIC UNIT

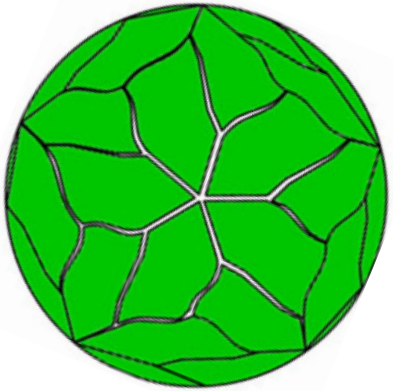


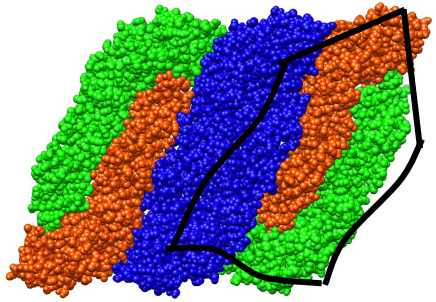


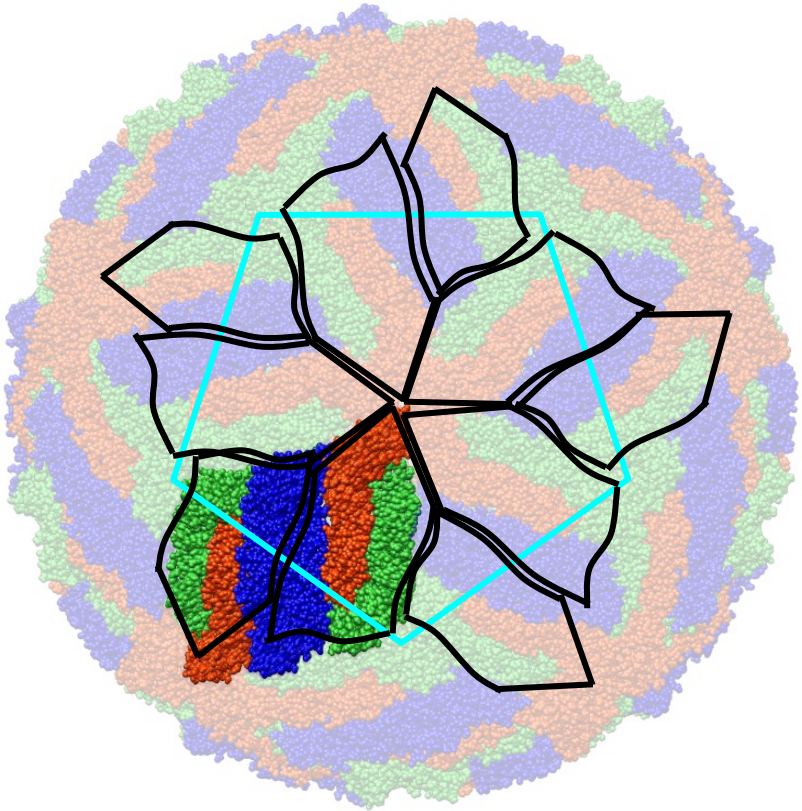


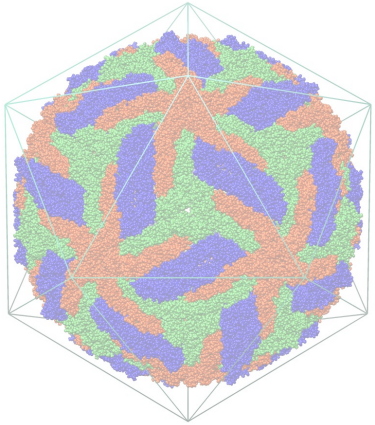


ENVELOPE SYMMETRY
ASYMMETRIC UNIT

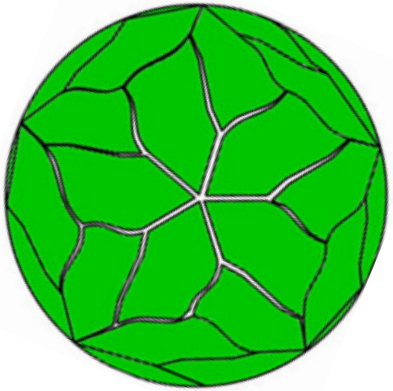


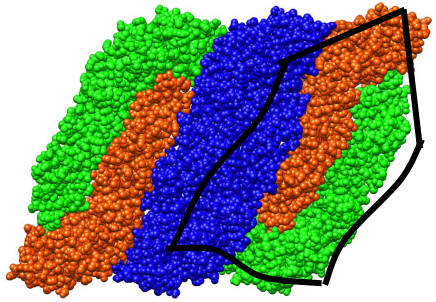


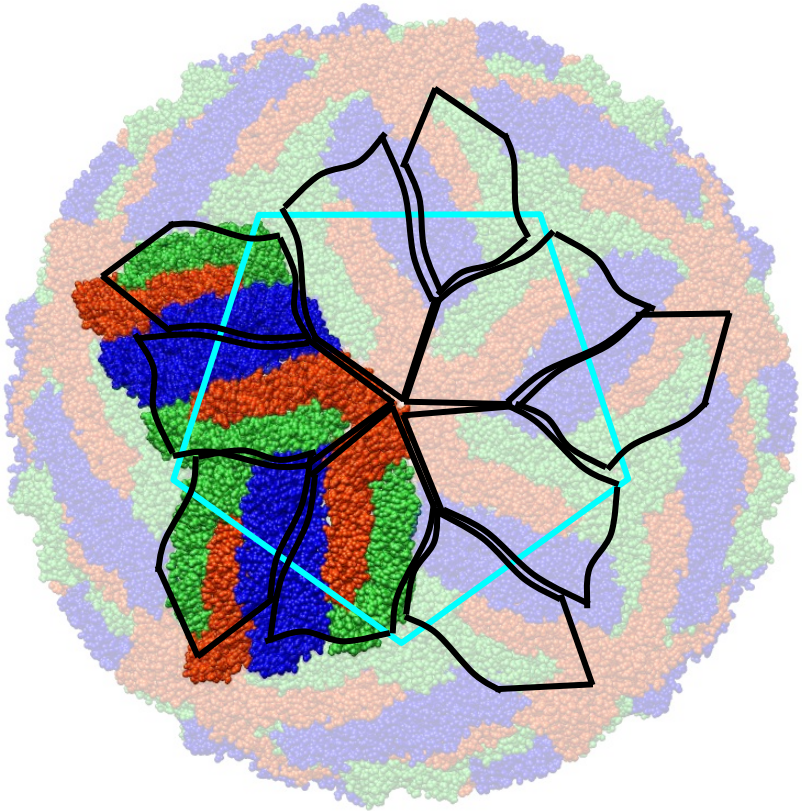


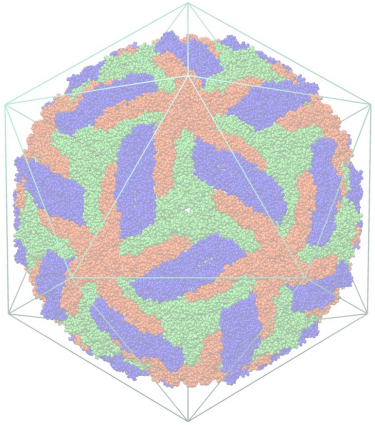


ENVELOPE SYMMETRY
ASYMMETRIC UNIT

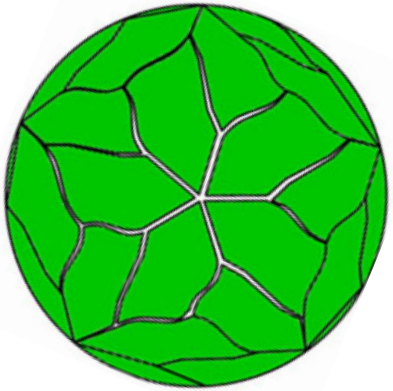


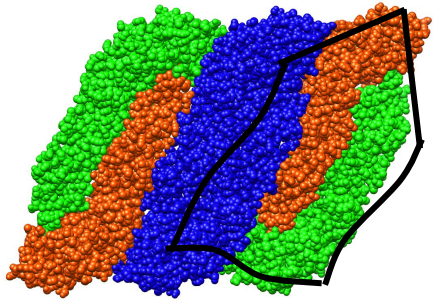


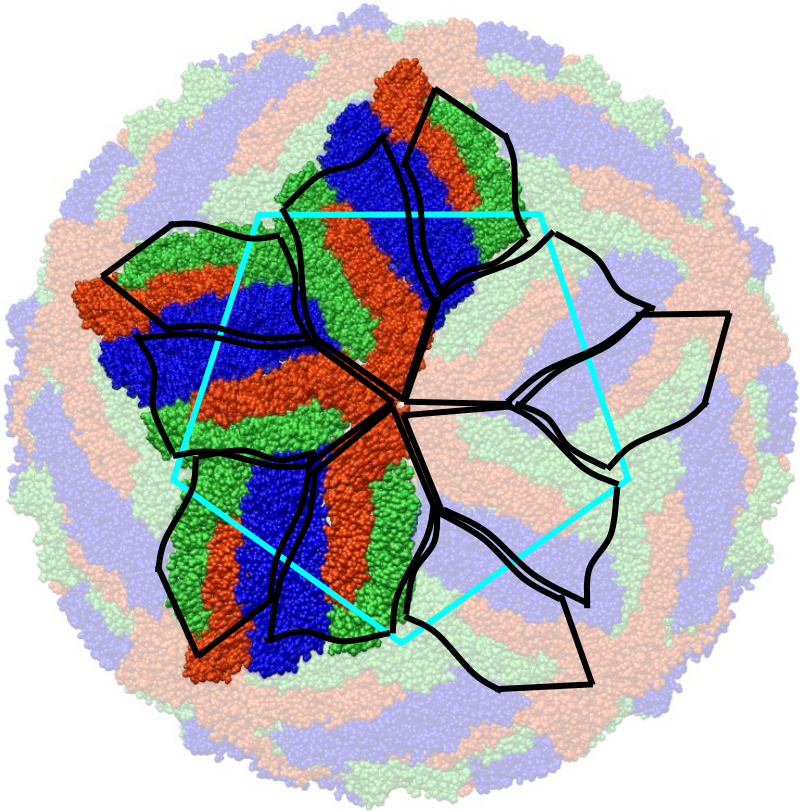


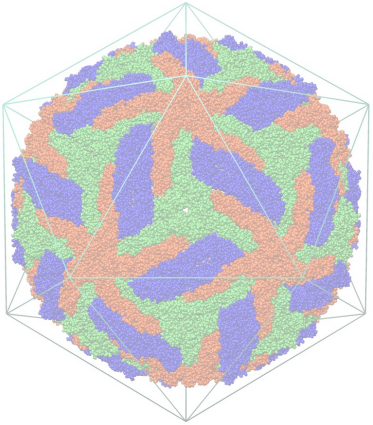


ENVELOPE SYMMETRY
ASYMMETRIC UNIT

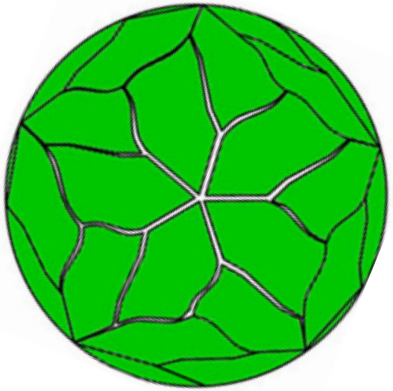


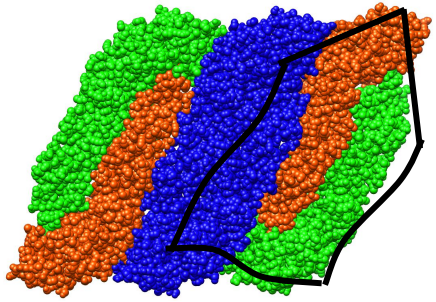


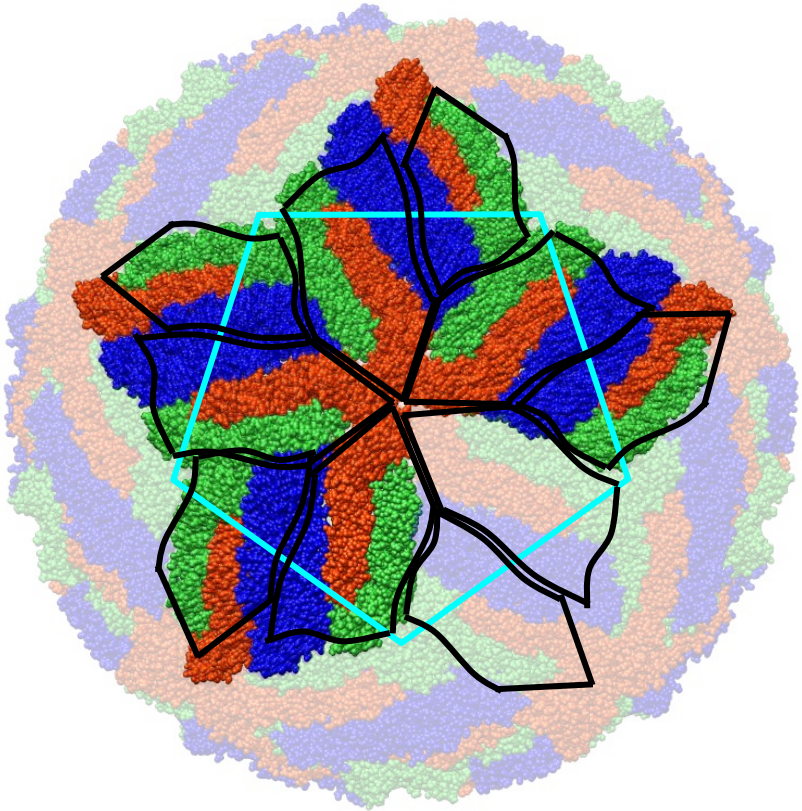


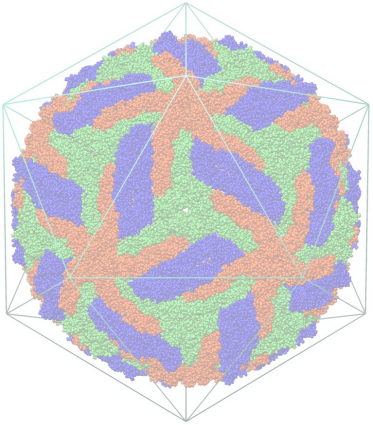


ENVELOPE SYMMETRY
ASYMMETRIC UNIT

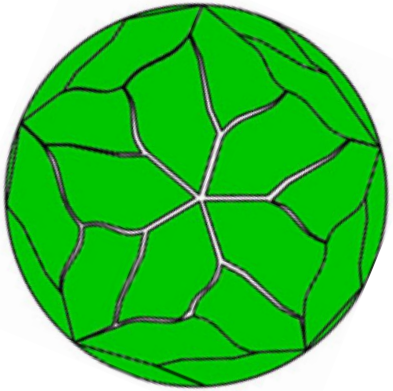


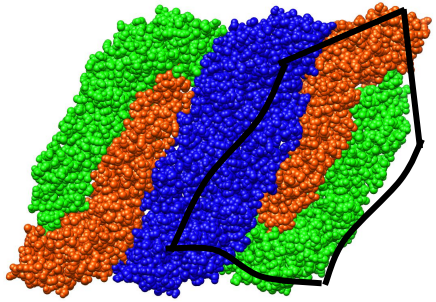


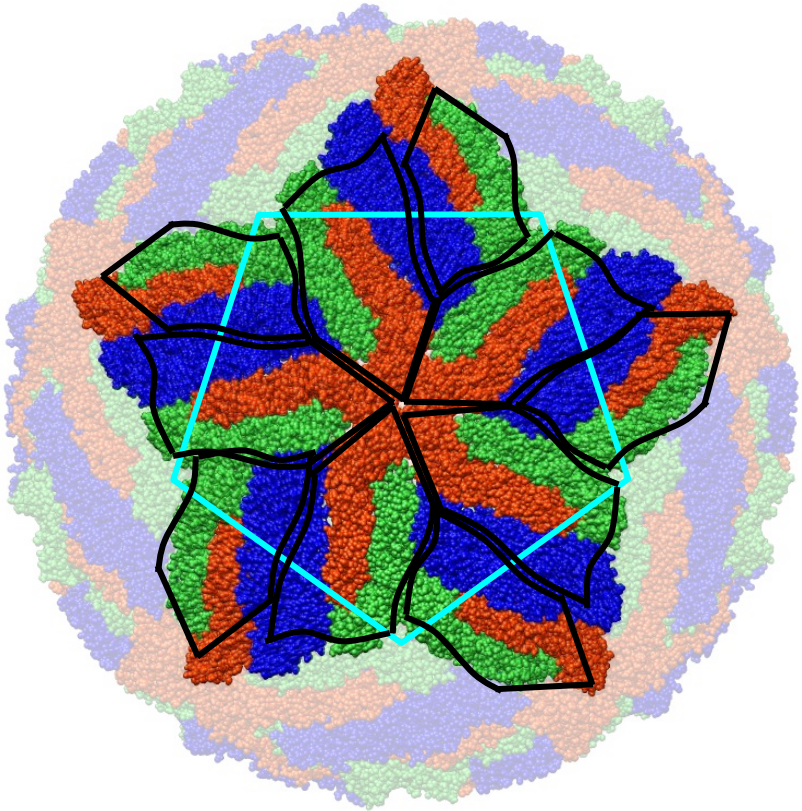


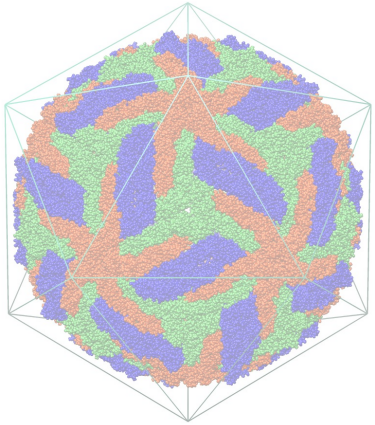


ENVELOPE SYMMETRY
ASYMMETRIC UNIT

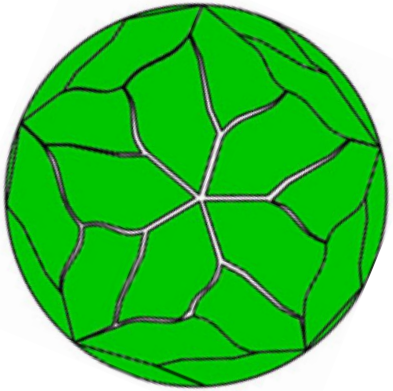


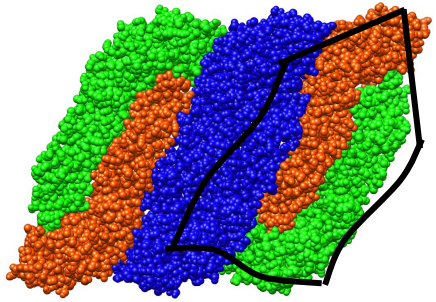


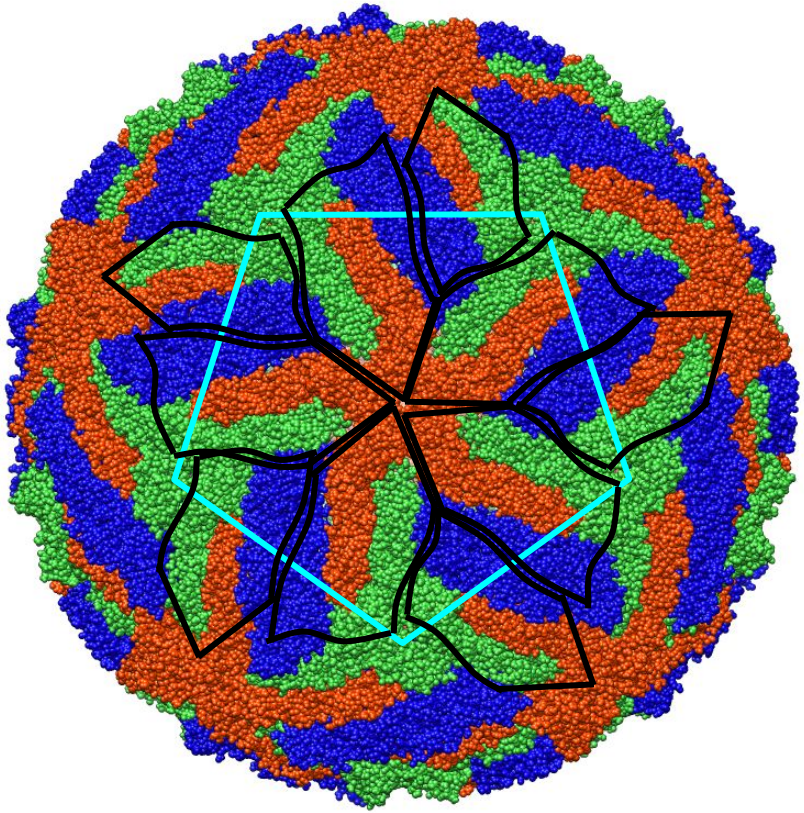


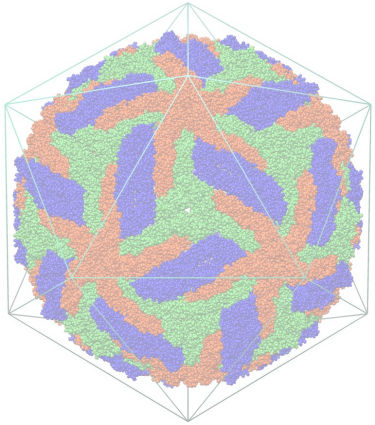


ENVELOPE SYMMETRY
ASYMMETRIC UNIT

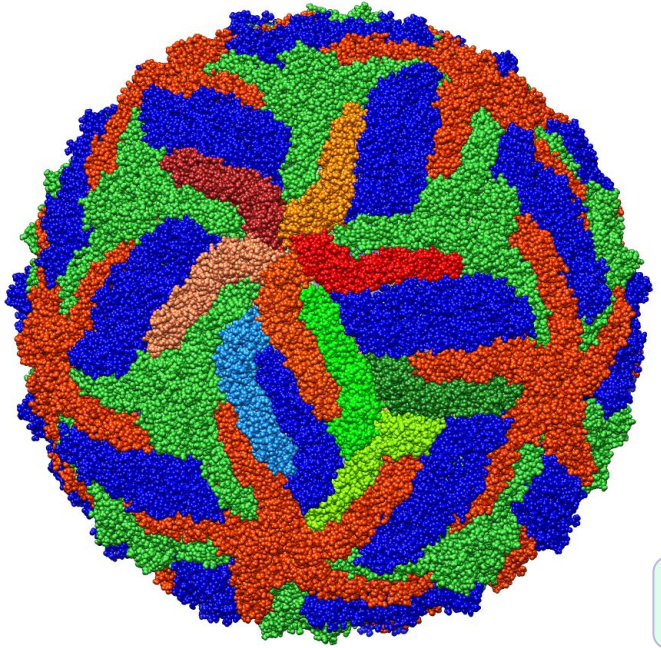


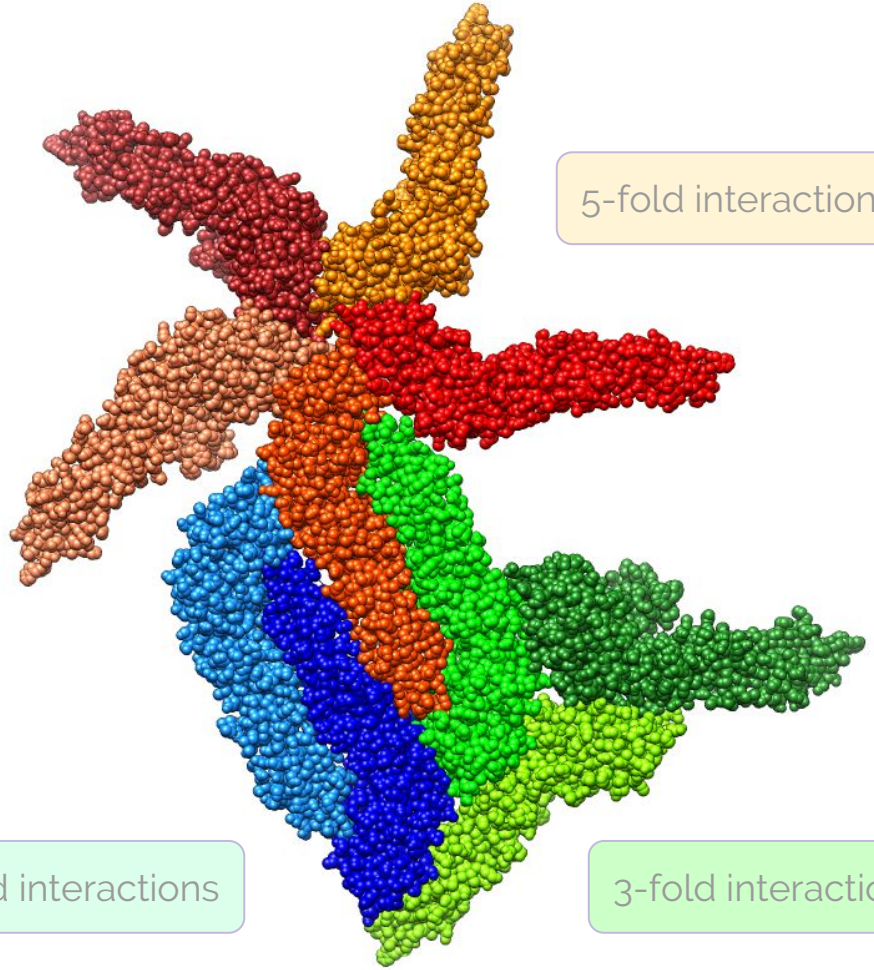






ENVELOPE SYMMETRY
INTERFACES



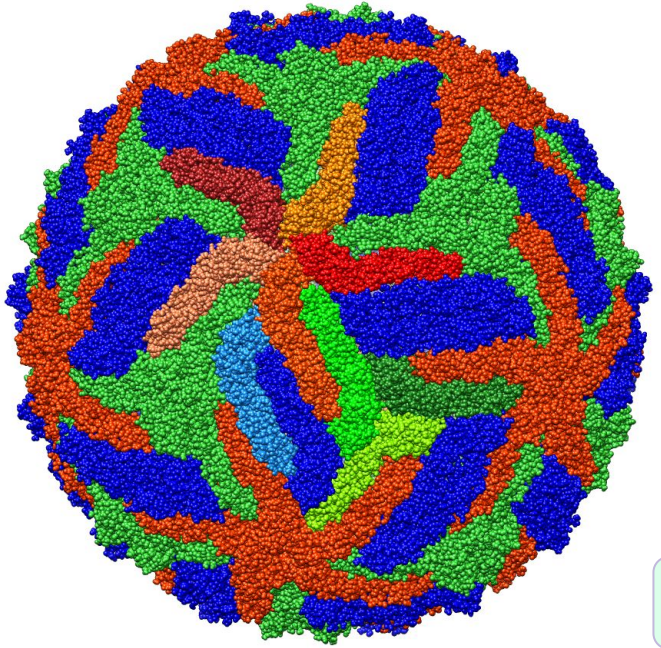


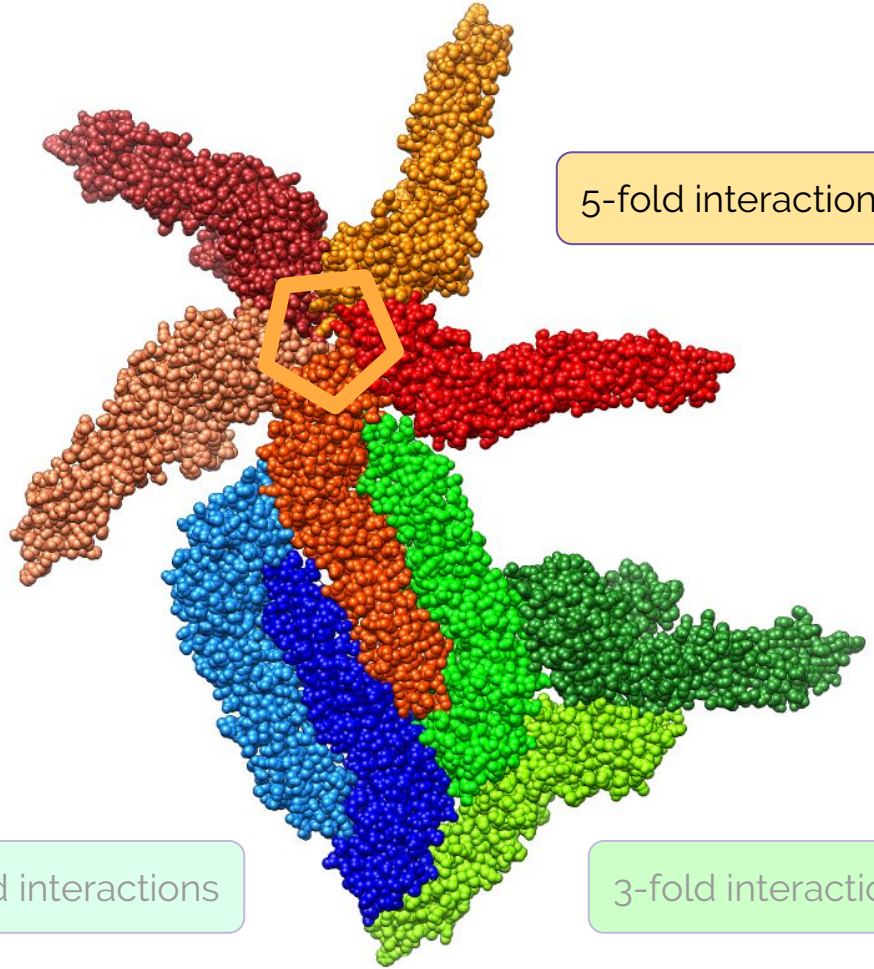
5-fold interactions

2-fold interactions

3-fold interactions

ENVELOPE SYMMETRY
INTERFACES



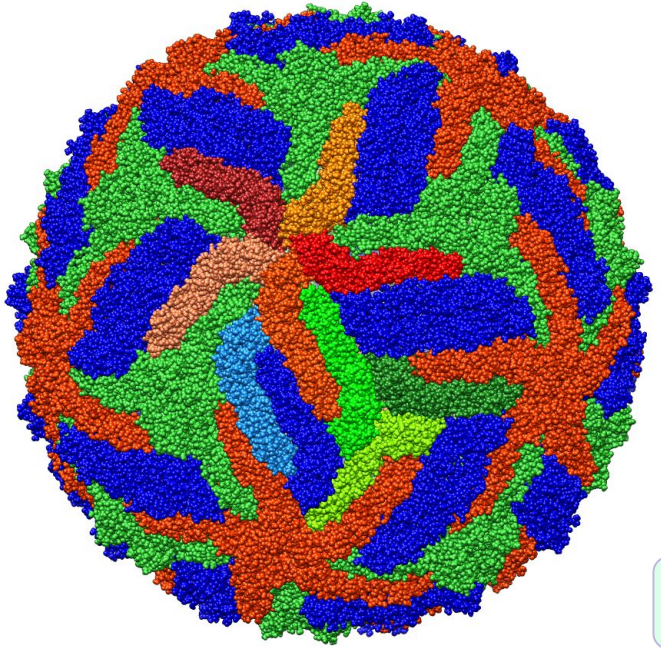


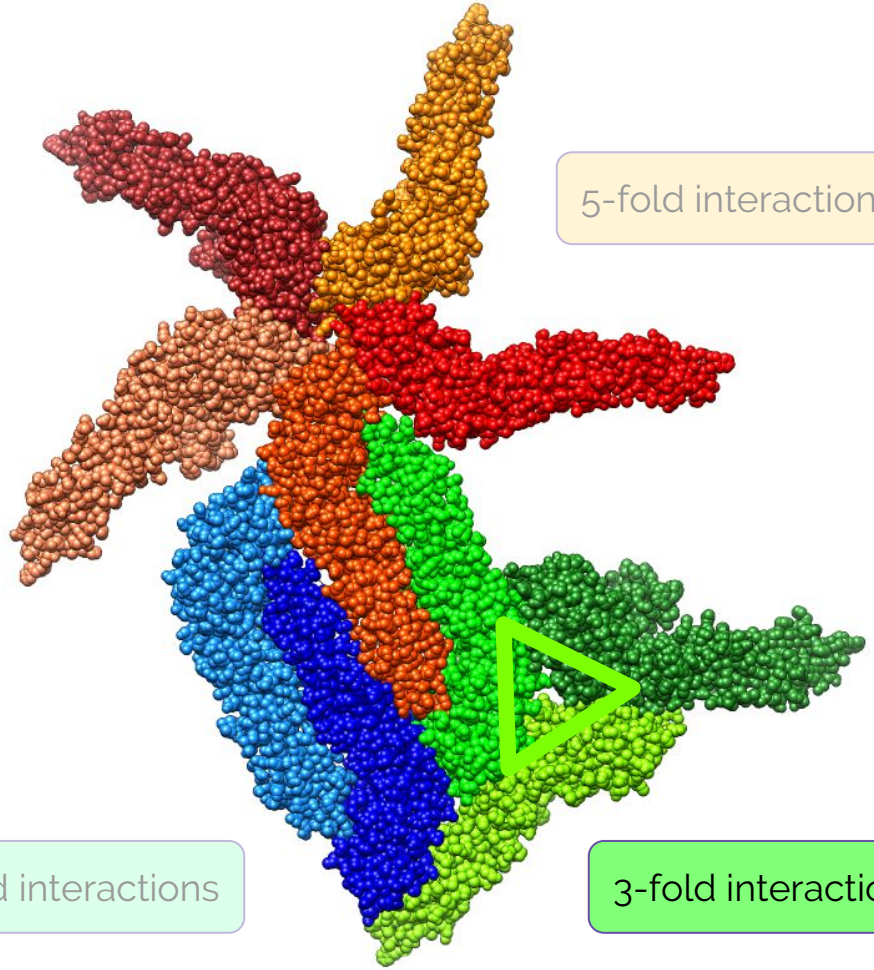
5-fold interactions

2-fold interactions

3-fold interactions

ENVELOPE SYMMETRY
INTERFACES



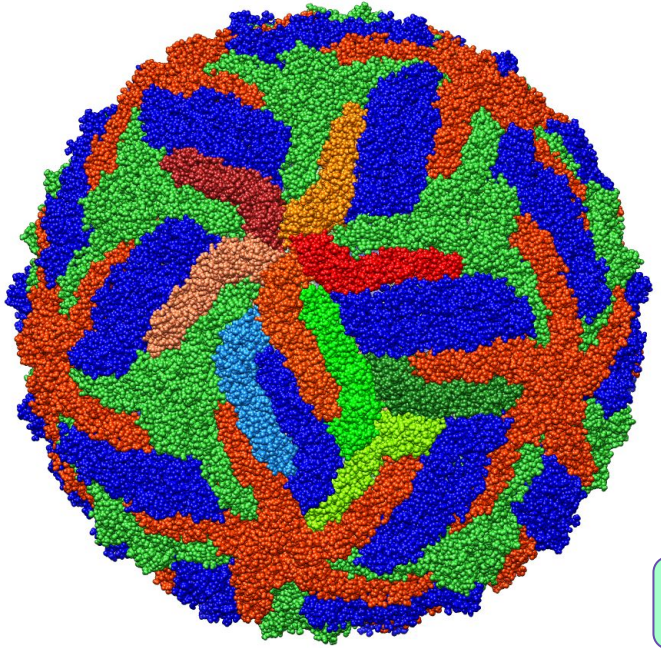


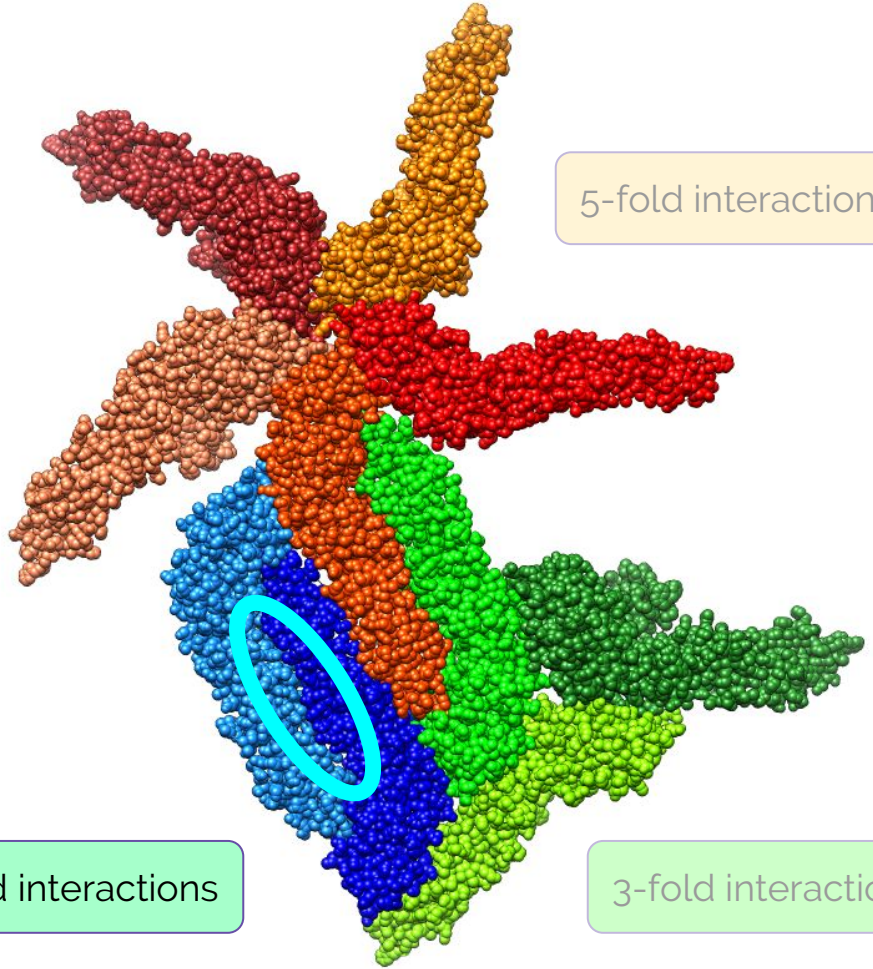
5-fold interactions

2-fold interactions

3-fold interactions

ENVELOPE SYMMETRY
INTERFACES





5-fold interactions

2-fold interactions

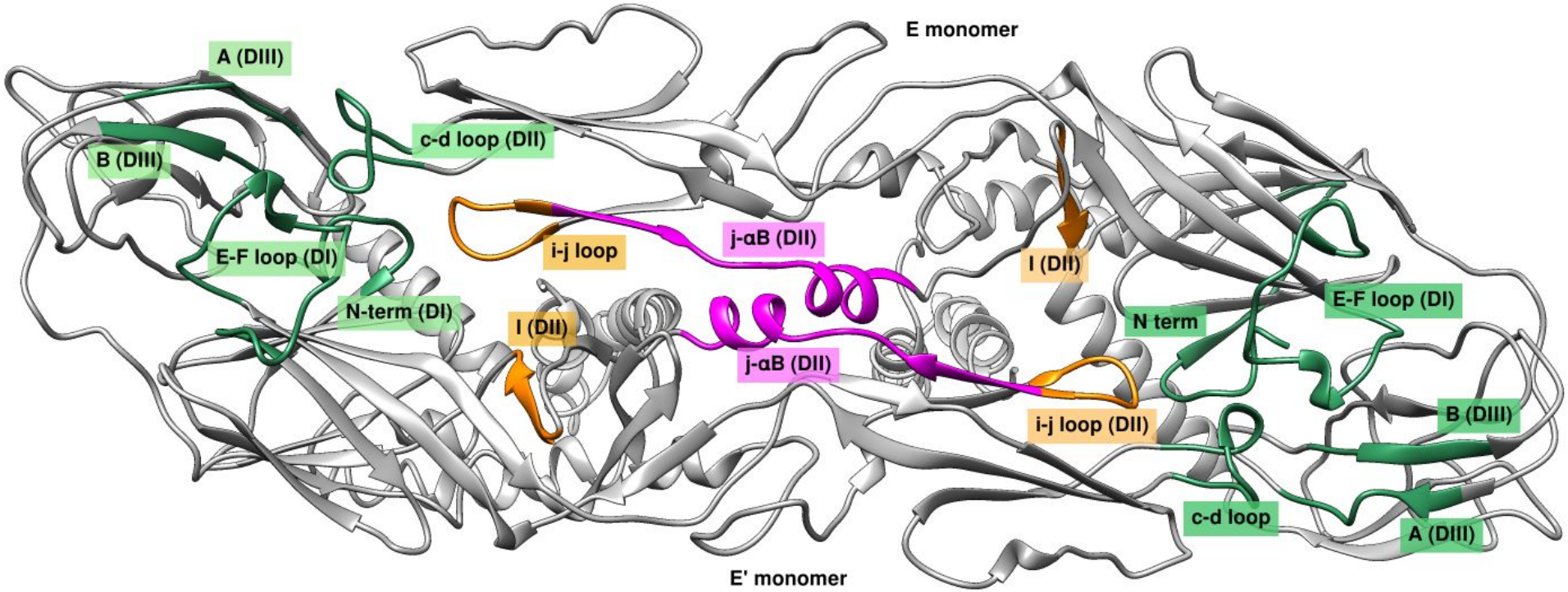
3-fold interactions

Interactions

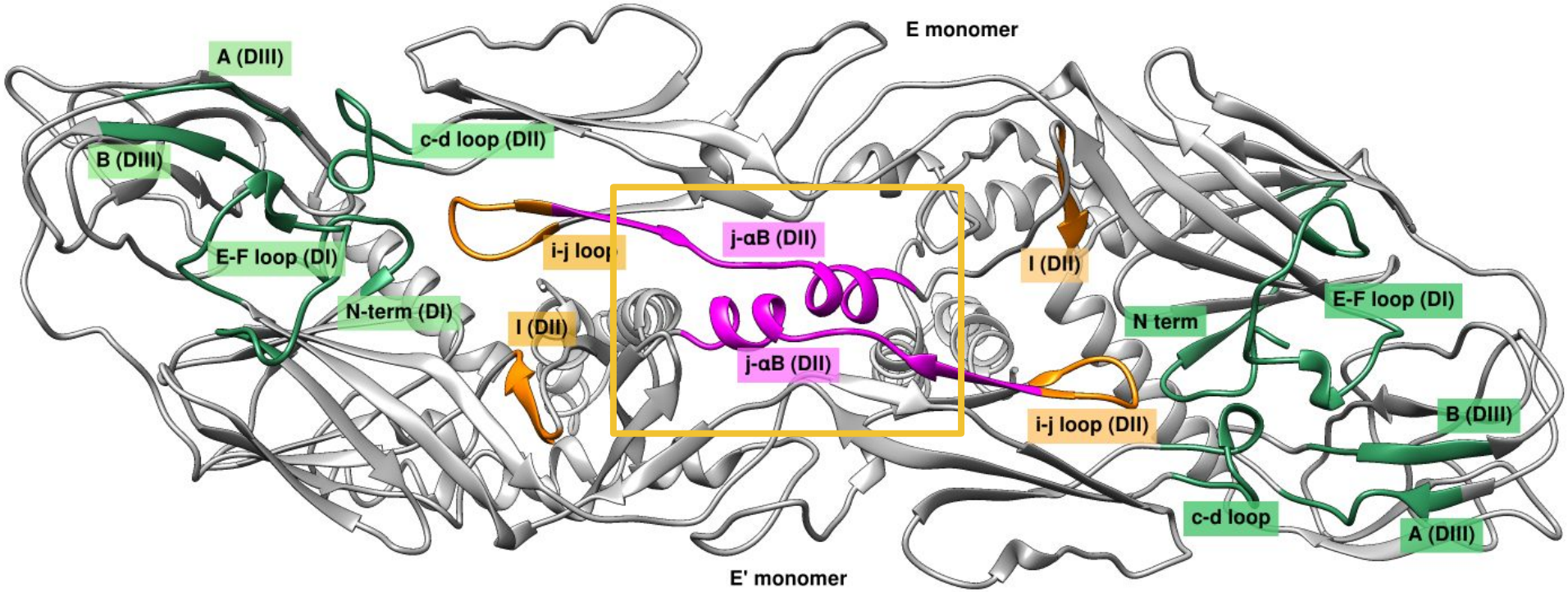
Interactions

2-fold interface

2-FOLD E PROTEIN AXIS E-E INTERACTIONS



2-FOLD E PROTEIN AXIS E-E POLAR INTERACTIONS



2-FOLD E PROTEIN AXIS E-E POLAR INTERACTIONS

```
Zika_virus_1/1-504                          KRQTVVVLGSQEGAVHTALAGALEAEMDGA---KGRLSSGHLKCRLKMDK 297
Zika_virus_2/1-498                          KRQTVVVLGSQEGAVHTALAGALEAEMDGA---KGRLFSGHLKCRLKMDK 291
Japanese_encephalitis_virus_1/1-500         TKQSVVALGSQEGGLHQALAGAIVVEYSS----SVKLTSGHLKCRLKMDK 293
Japanese_encephalitis_virus_2/1-499         TKQSVVALGSQEGSLHQALAGAIVVEYSS----SVKLTSGHLKCRLKMDK 292
West_Nile_virus_1/1-501                     TKQSVIALGSQEGALHQALAGAIPVEFSSN---TVKLTSGHLKCRVKLEK 294
West_Nile_virus_2/1-501                     TKQSVVALGSQEGALHQALAGAIPVEFSSN---TVKLTSGHLKCRVKMEK 294
Dengue_virus_4_1/1-495                      KRQDVTVLGSQEGAMHSALAGATEVDSGD----GNHMFAGHLKCKVRMEK 291
Dengue_virus_4_2/1-495                      KRQDVTVLGSQEGAMHSALAGATEVDSGD----GNHMFAGHLKCKVRMEK 291
Dengue_virus_1_1/1-495                      KKQEVVVLGSQEGAMHTALTGATEIQTSG----TTTIFAGHLKCRLKMDK 291
Dengue_virus_1_2/1-495                      KKQEVVVLGSQEGAMHTALTGATEIQTSG----TTTIFAGHLKCRLKMDK 291
Dengue_virus_3_1/1-493                      KKQEVVVLGSQEGAMHTALTGATEIQTSG----GTSIFAGHLKCRLKMDK 289
Dengue_virus_3_2/1-493                      KKQEVVVLGSQEGAMHTALTGATEIQNSG----GTSIFAGHLKCRLKMDK 289
Dengue_virus_2_1/1-495                      KKQDVVVLGSQEGAMHTALTGATEIQMSS----GNLLFTGHLKCRLRMDK 291
Dengue_virus_2_2/1-495                      KKQDVVVLGSQEGAMHTALTGATEIQMSS----GNLLFTGHLKCRLRMDK 291
Yellow_fever_virus_1/1-493                  ATIKVLALGNQEGSLKTALTGAMRVTKDTNNSKLYKLHGGHVACRVKLSA 289
Yellow_fever_virus_2/1-493                  ATIKVLALGNQEGSLKTALTGAMRVTKDTNNSKLYKLHGGHVACRVKLSA 289
                                             * .**.****.::  **:**     .  .     :  **: *::::.
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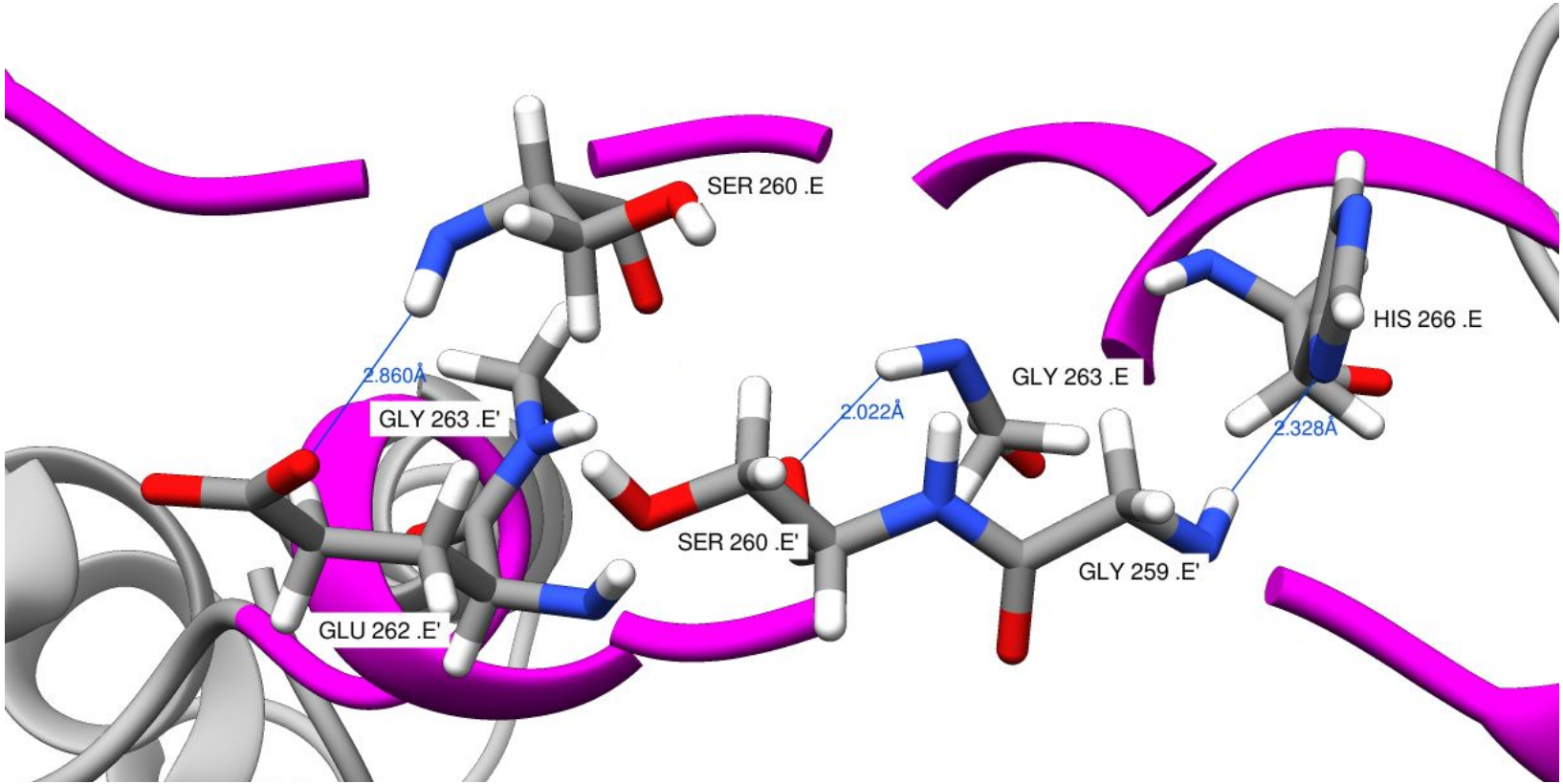
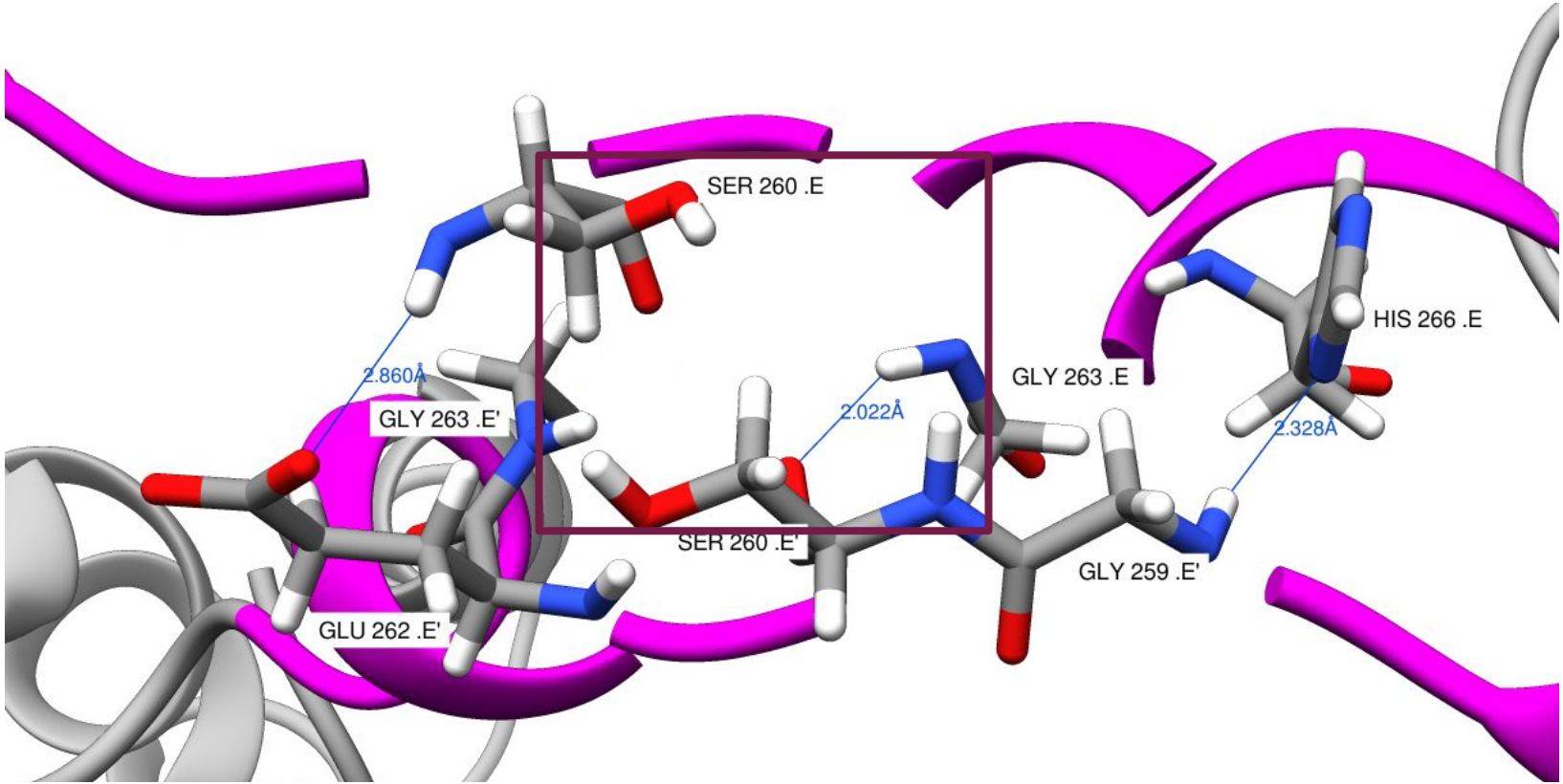
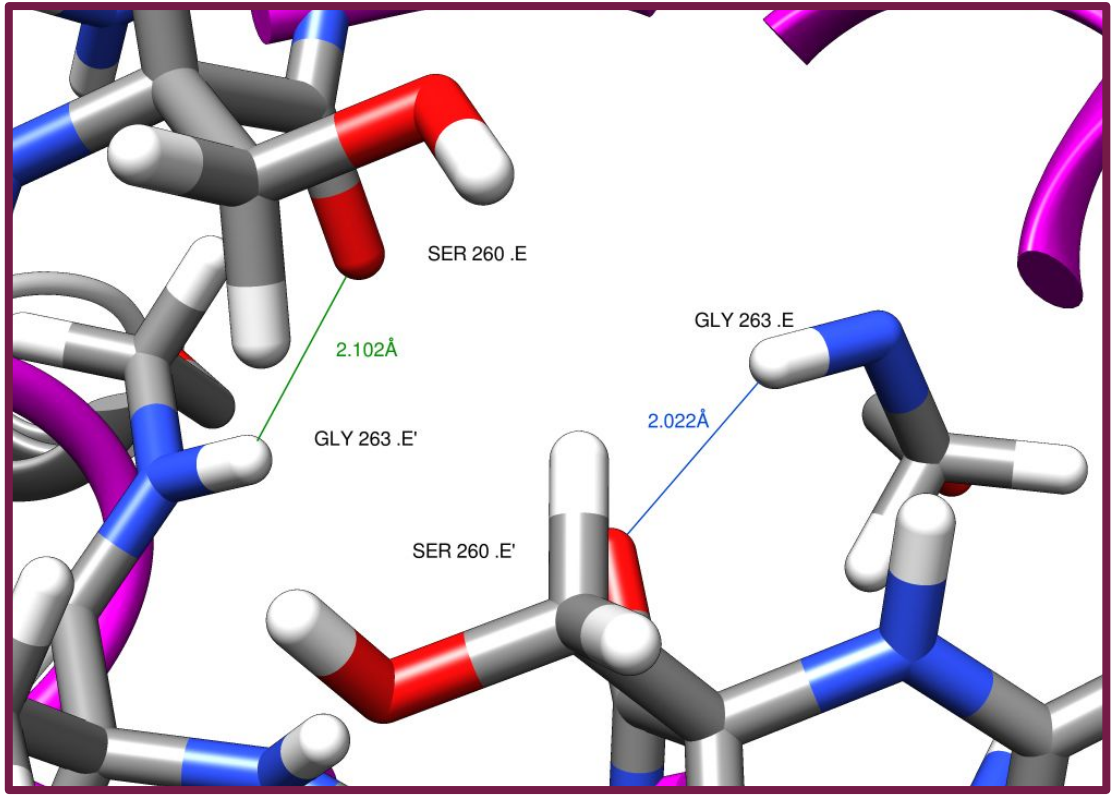
2-FOLD E PROTEIN AXIS E-E POLAR INTERACTIONS



2-FOLD E PROTEIN AXIS E-E POLAR INTERACTIONS

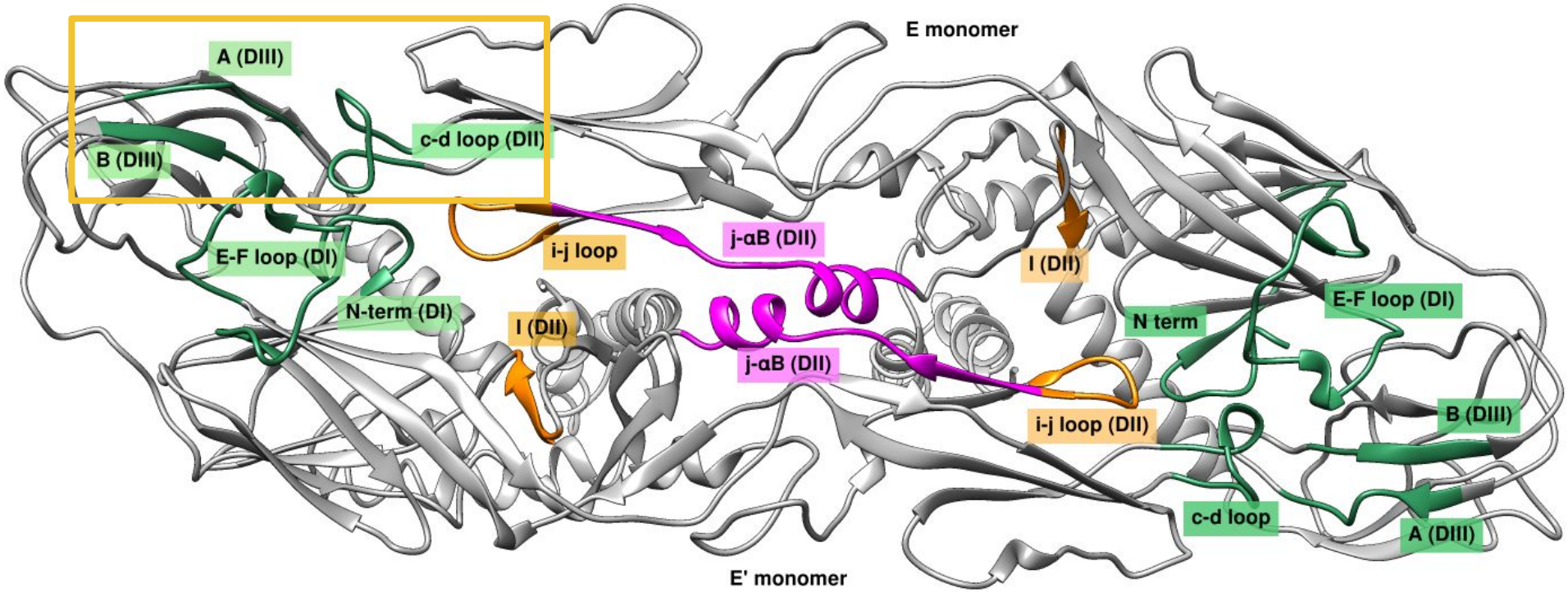


2-FOLD AXIS E PROTEIN E-E POLAR INTERACTIONS

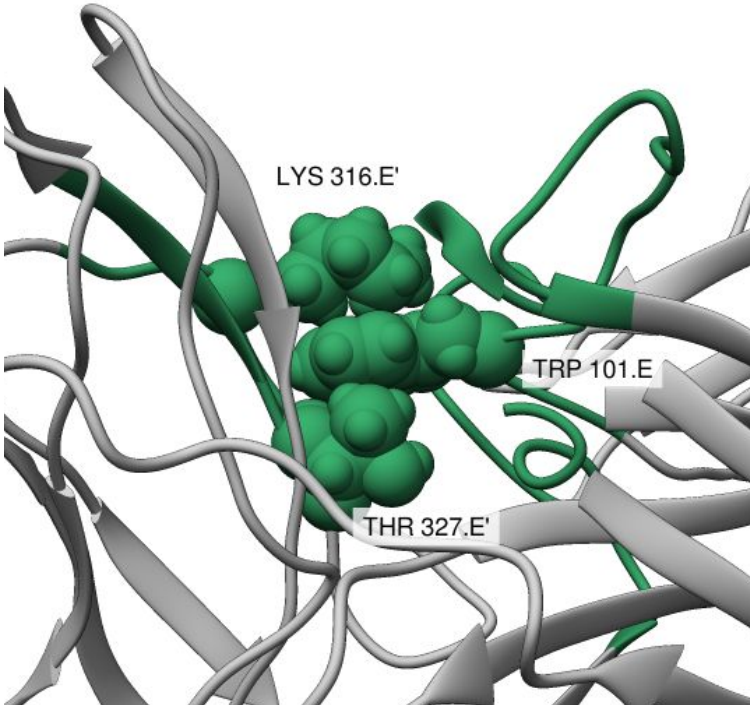


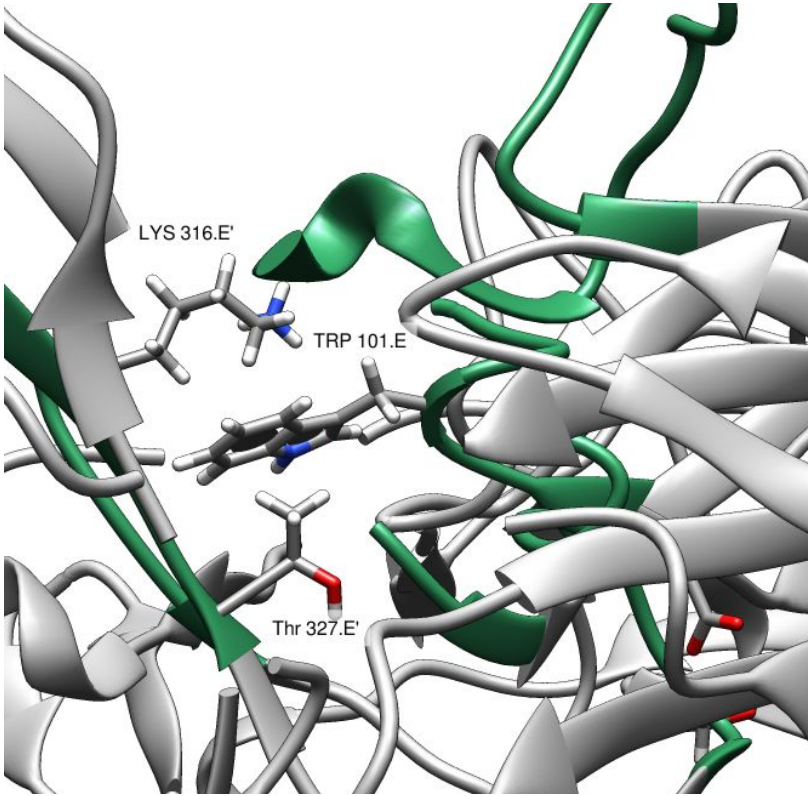
Example of Distorted Hbond

2-FOLD E PROTEIN AXIS E-E HYDROPHOBIC INTERACTIONS



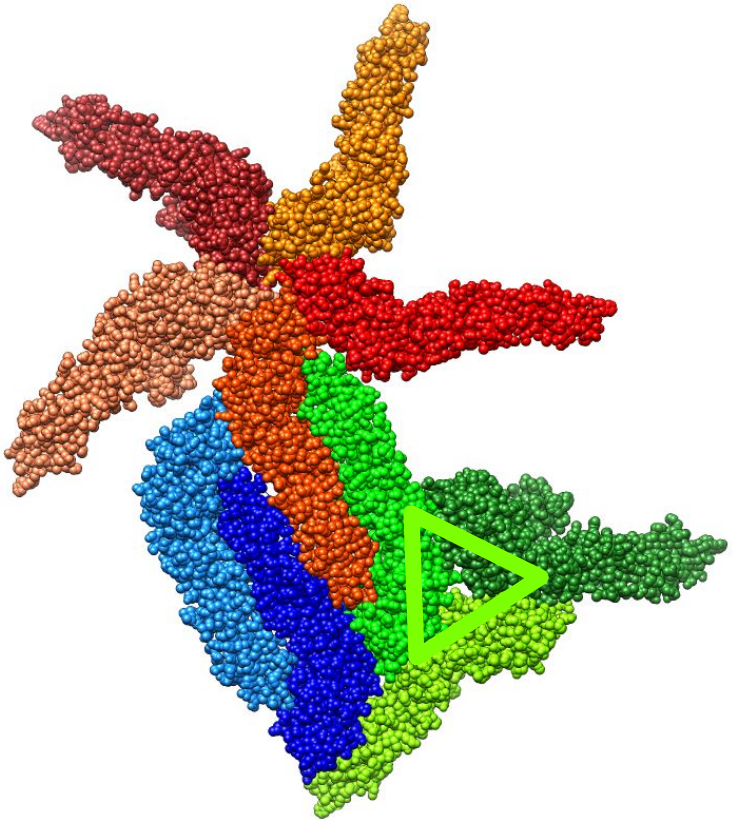
2-FOLD E PROTEIN AXIS E-E HYDROPHOBIC INTERACTIONS



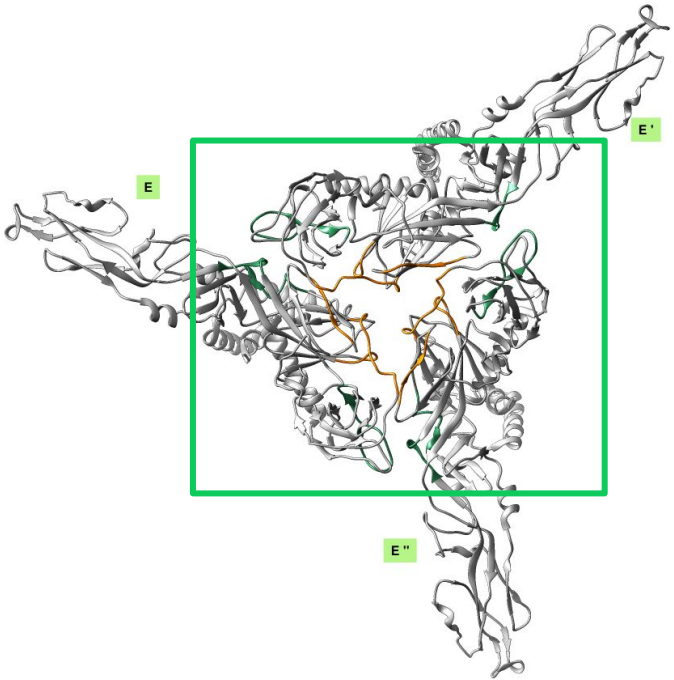


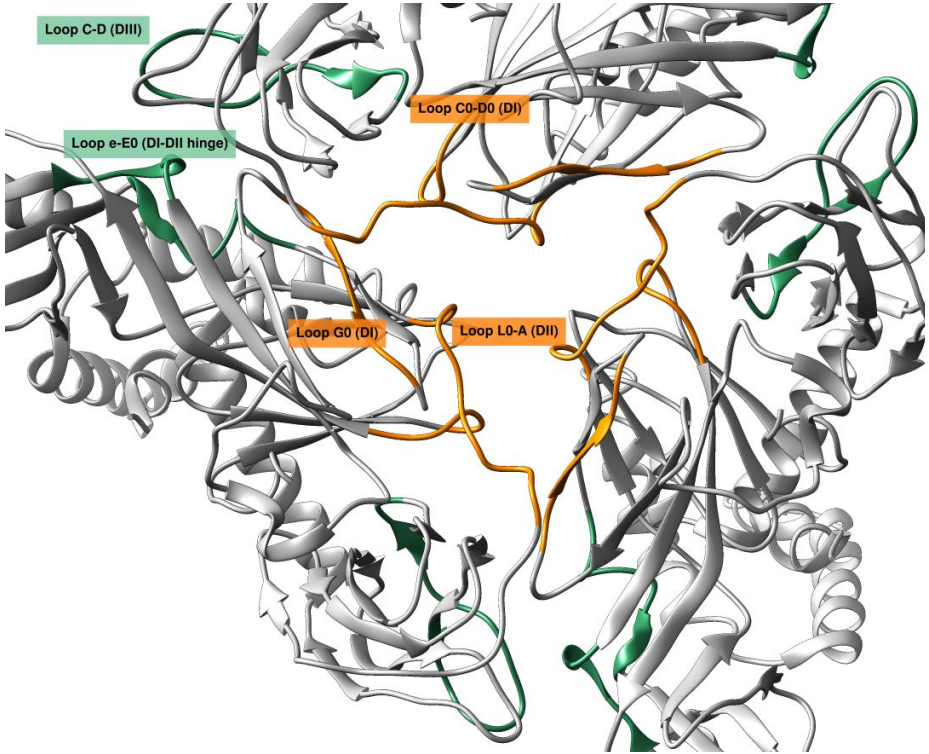
Interactions

3-fold interface

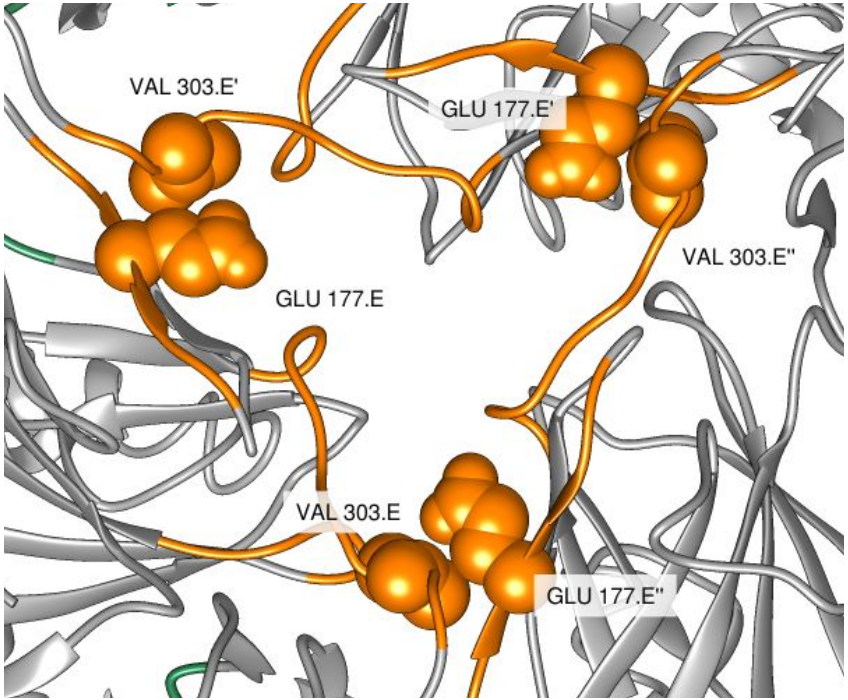


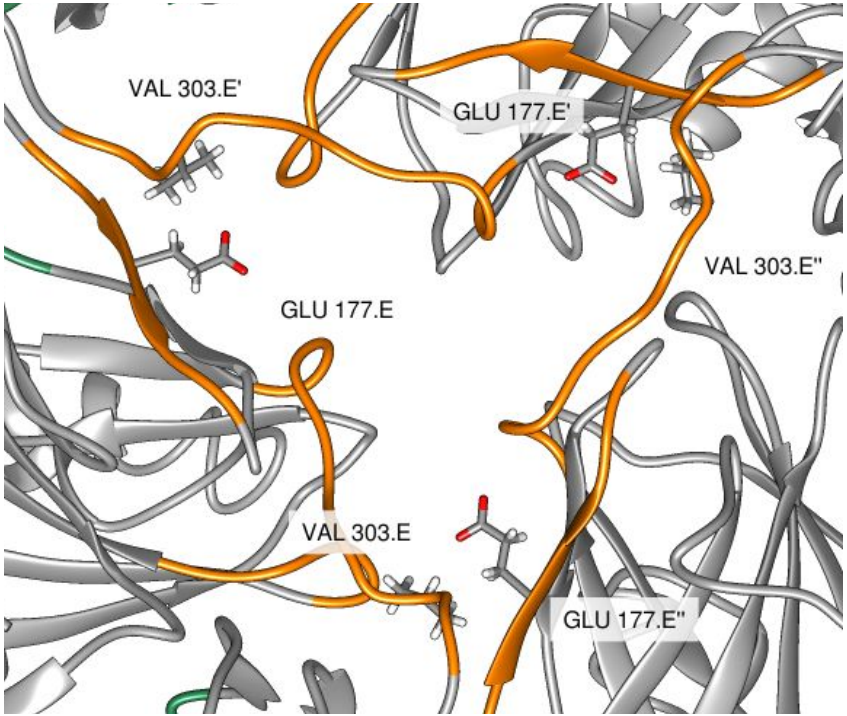
3-FOLD AXIS E PROTEIN E-E INTERACTIONS



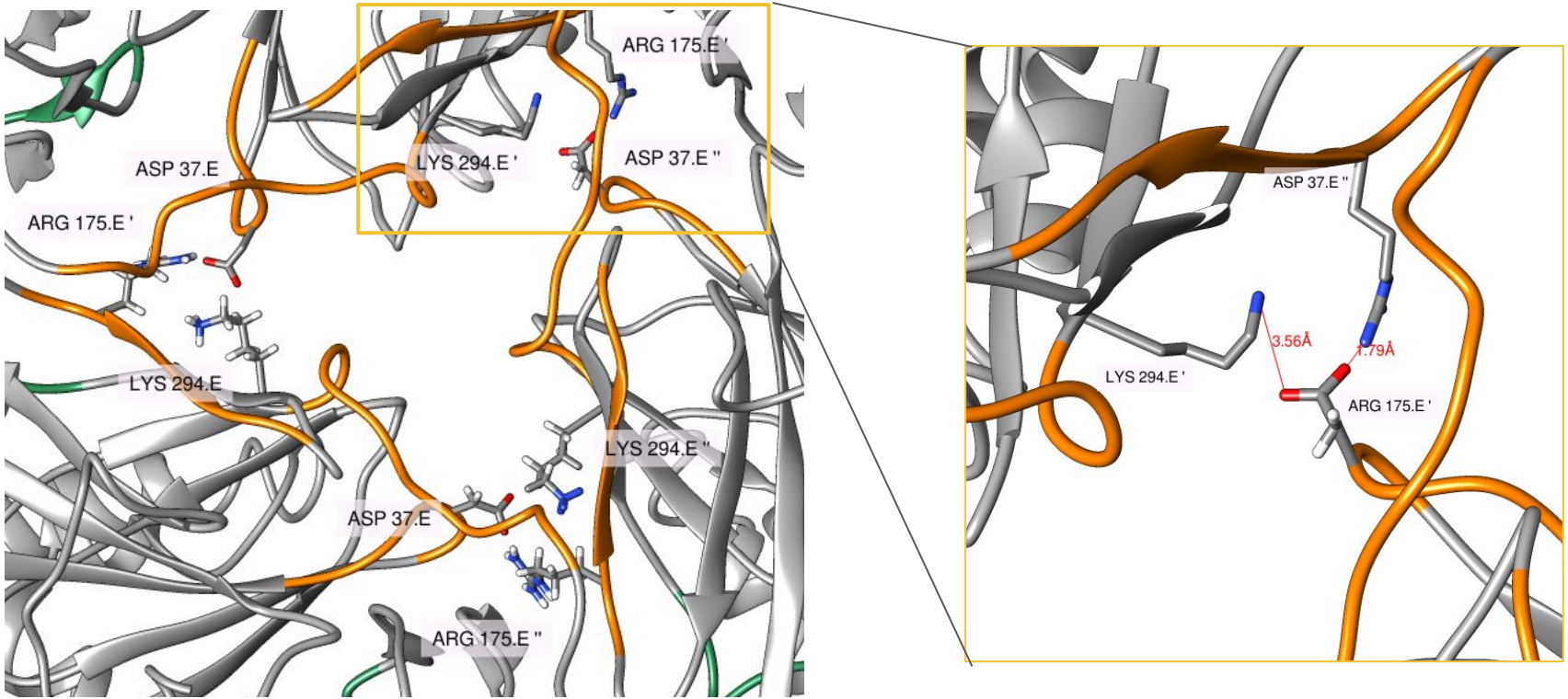


3-FOLD E PROTEIN AXIS E-E HYDROPHOBIC INTERACTIONS





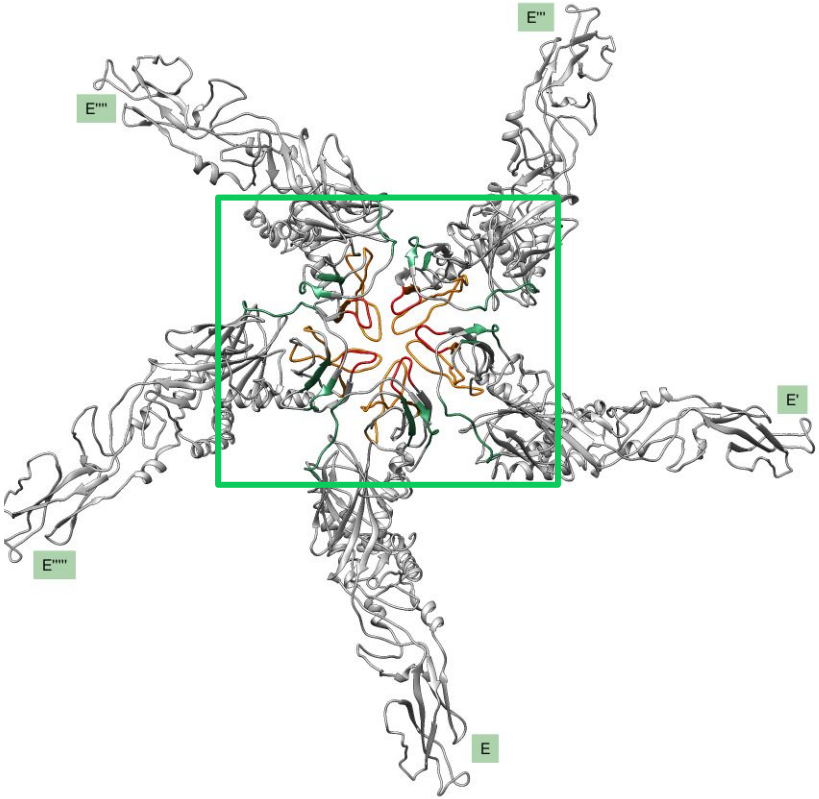
3-FOLD E PROTEIN AXIS E-E ELECTROSTATIC INTERACTIONS

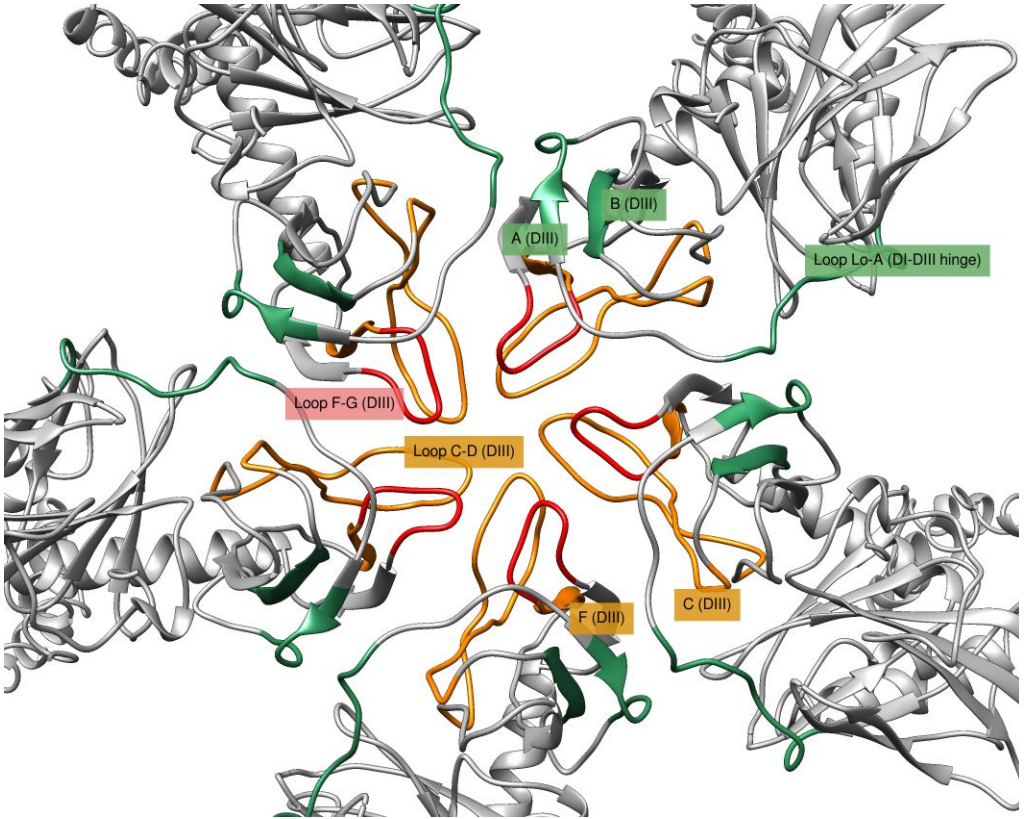


Interactions

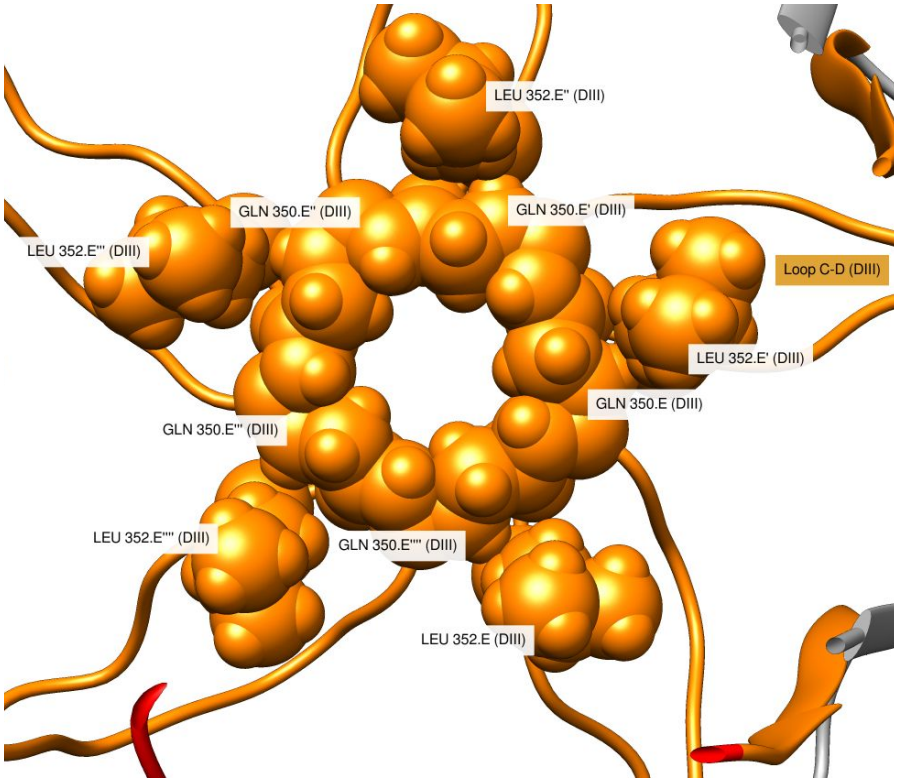
5-fold interface

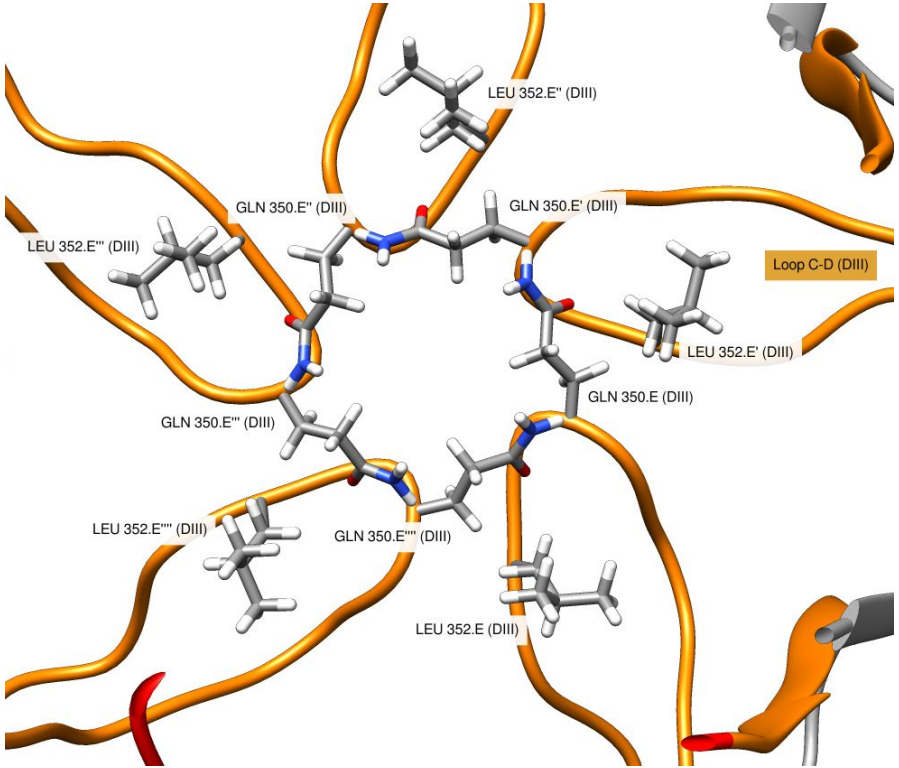
5-FOLD E PROTEIN AXIS E-E INTERACTIONS



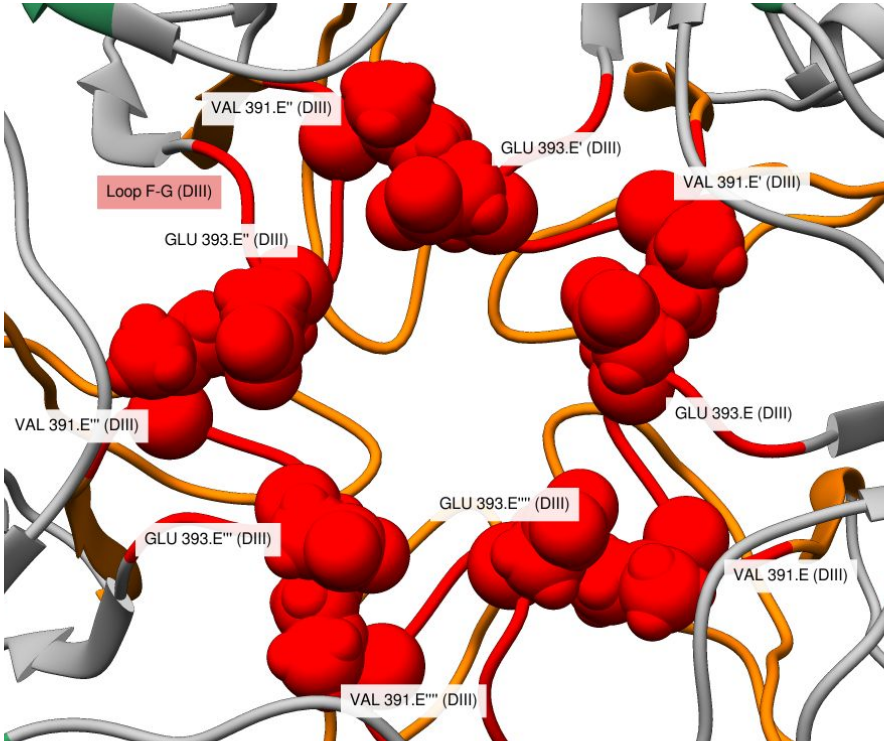


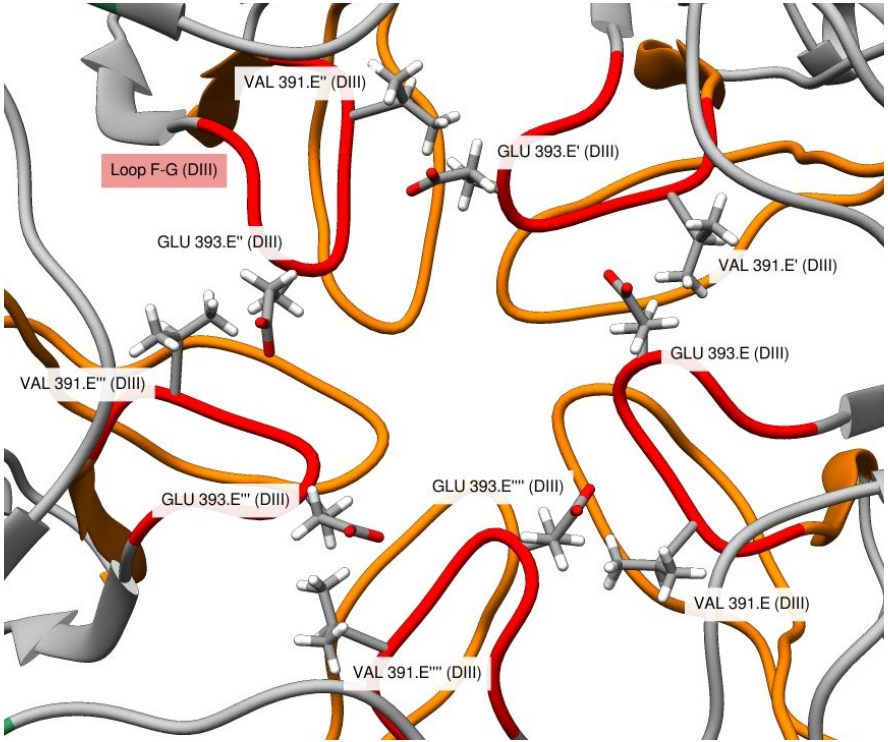
5-FOLD E PROTEIN AXIS E-E HYDROPHOBIC INTERACTIONS (LOOP C-D)



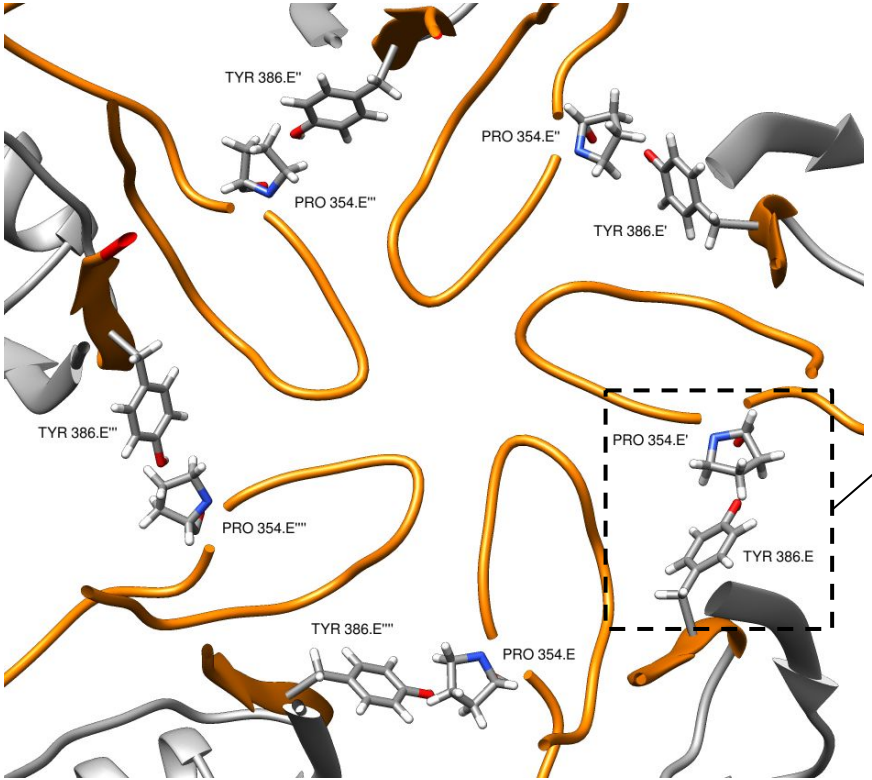


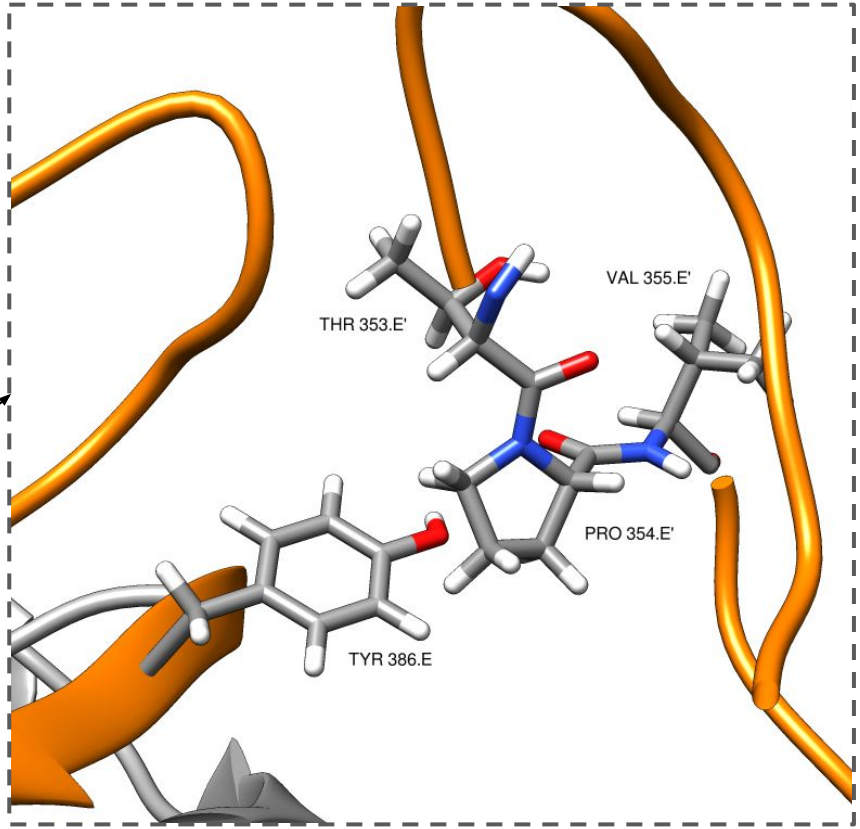
5-FOLD E PROTEIN AXIS E-E HYDROPHOBIC INTERACTIONS (LOOP F-G)



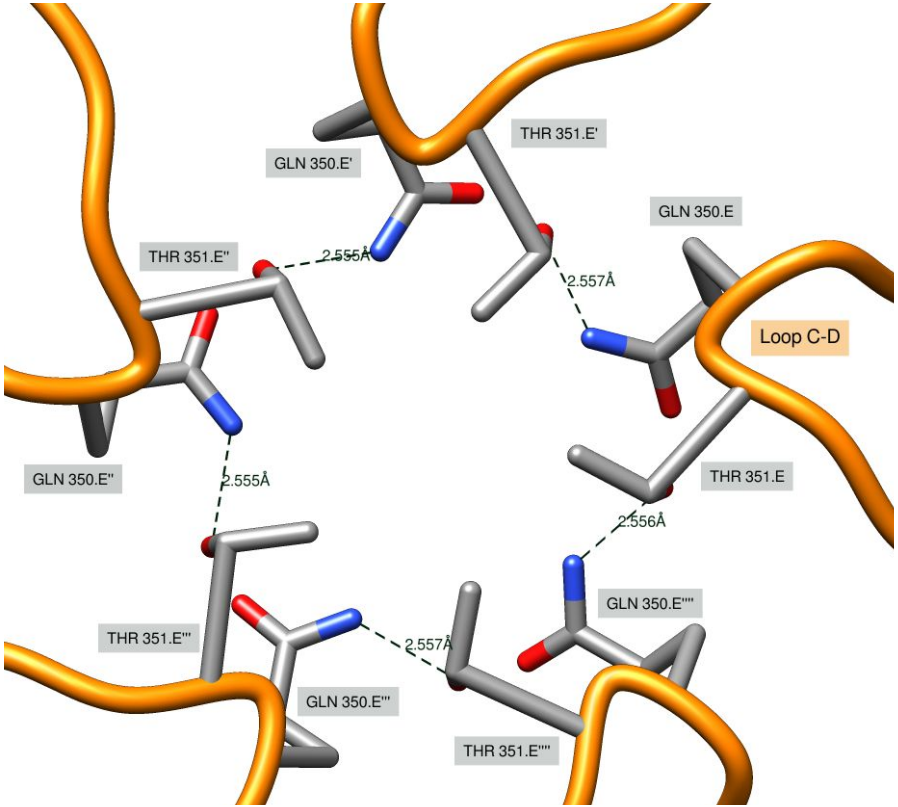


5-FOLD E PROTEIN AXIS E-E POLAR INTERACTIONS (LOOP C-D)

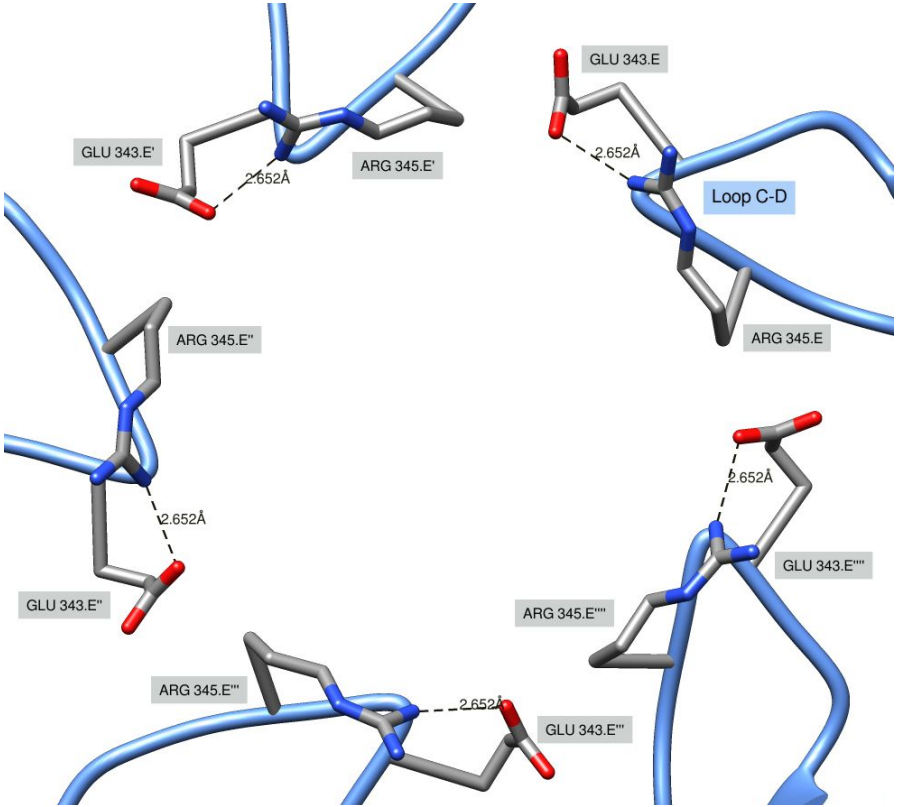




5-FOLD E PROTEIN AXIS E-E POLAR INTERACTIONS (LOOP C-D): ZIKV AND DENV 5-FOLD VERTEX COMPARISON



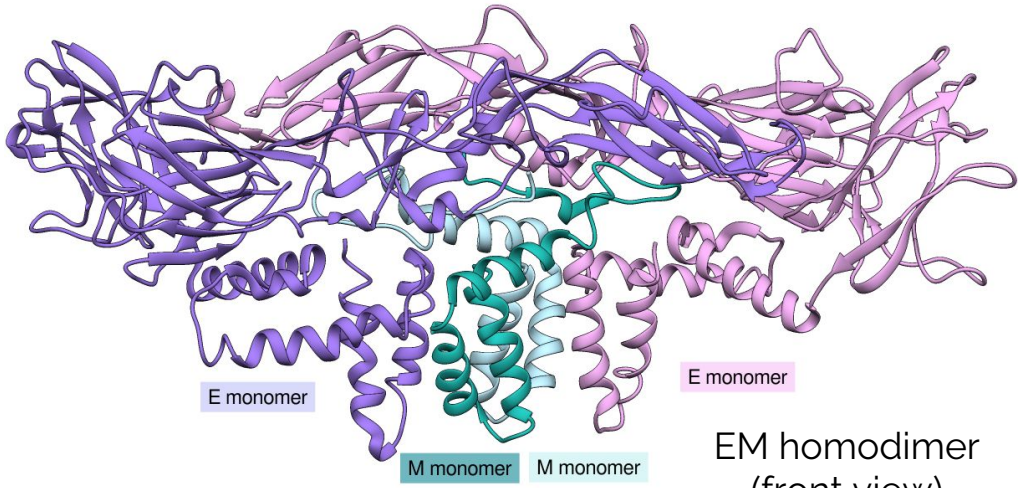
ZIKV



DENV

Interactions

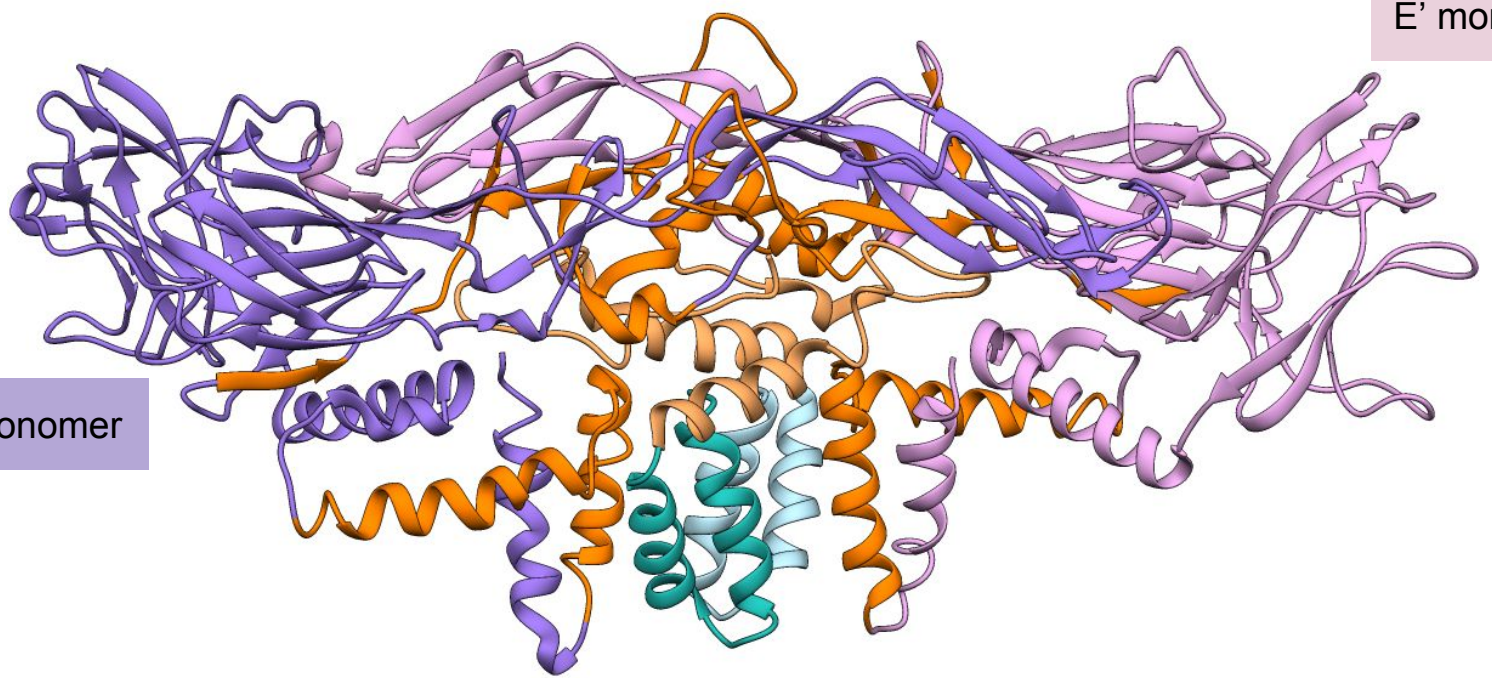
E-M interactions



EM homodimer
(front view)

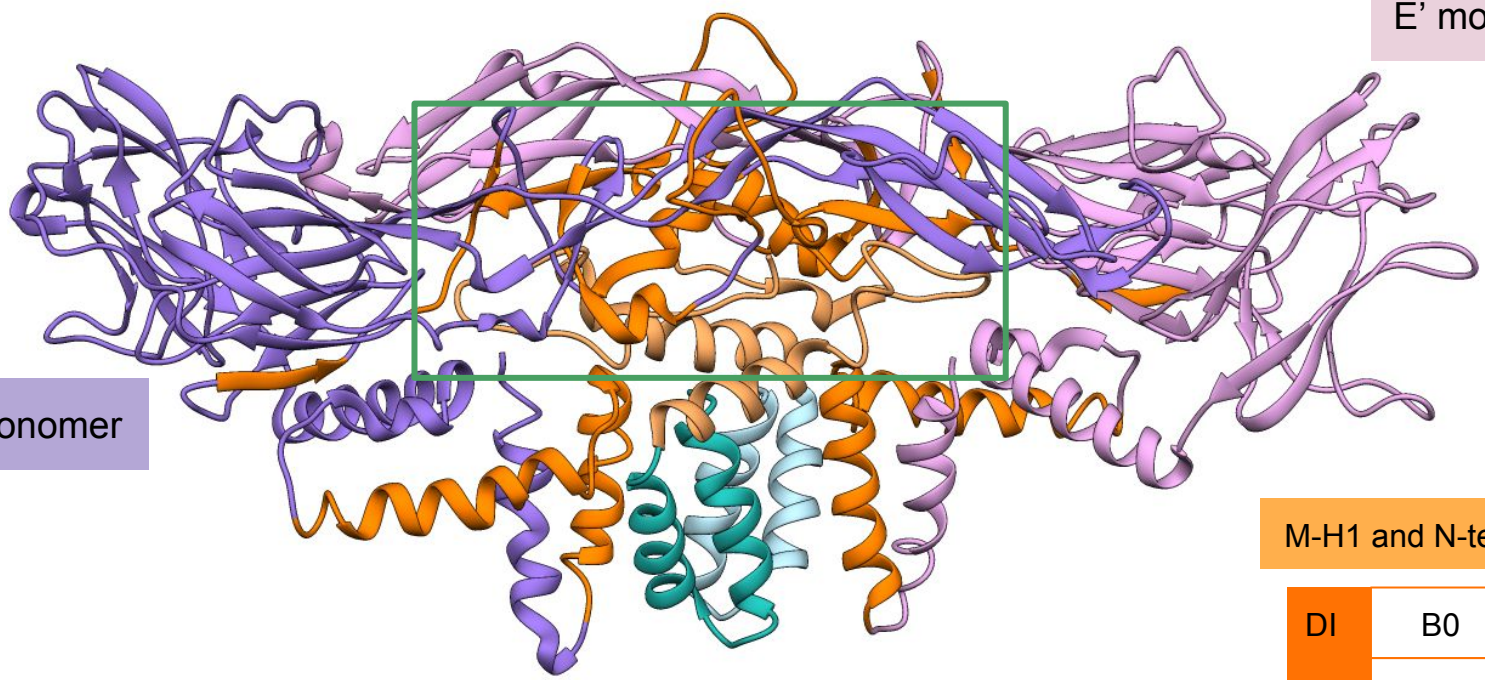
E-M INTERACTIONS

INTERACTION REGIONS

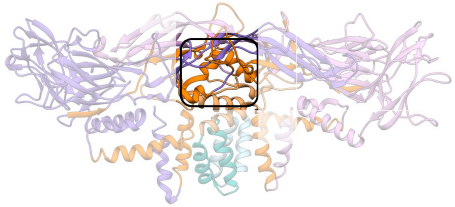


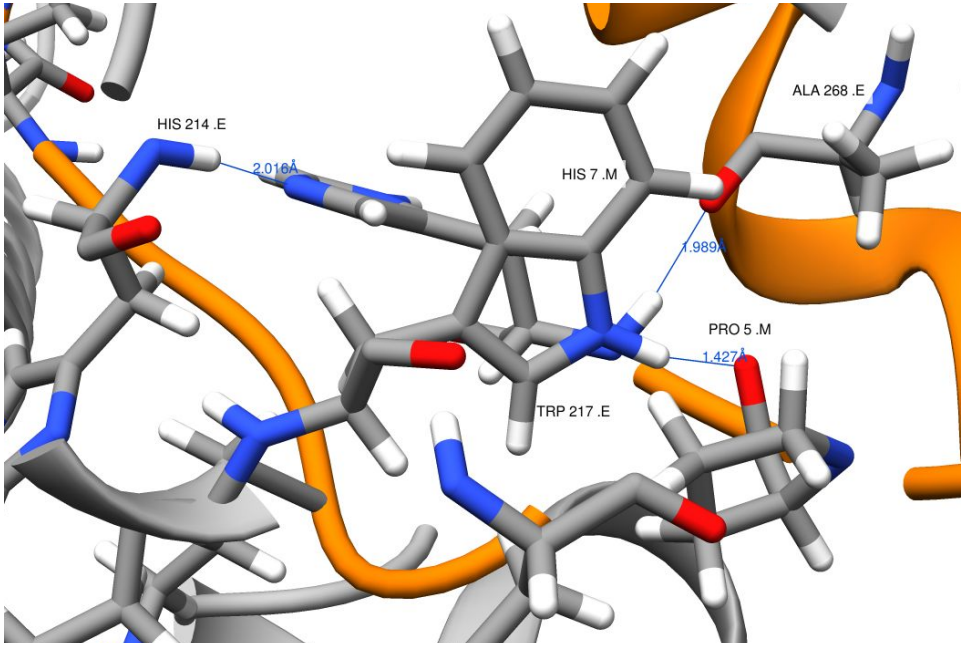
E-M INTERACTIONS

INTERACTION REGIONS

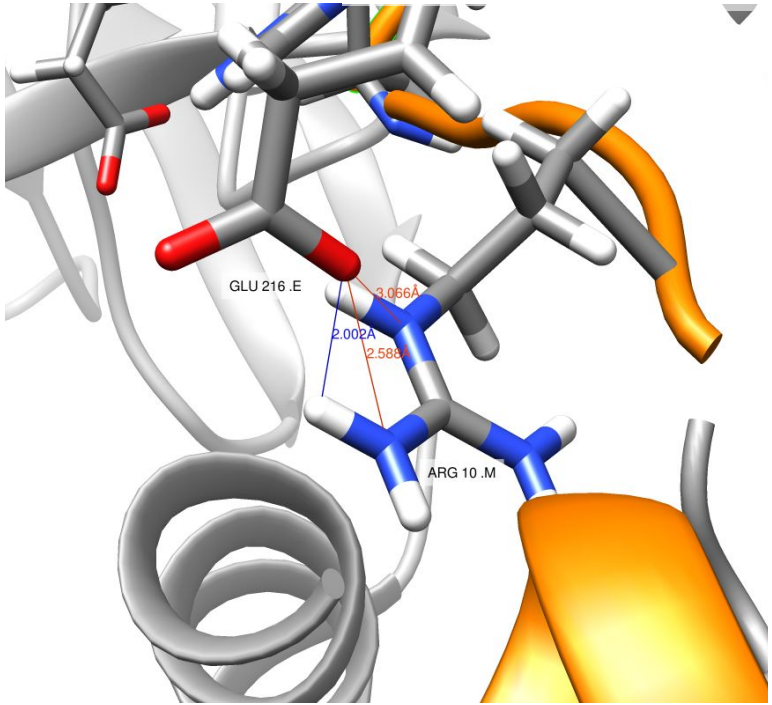


EM PROTEINS
E-M POLAR INTERACTIONS



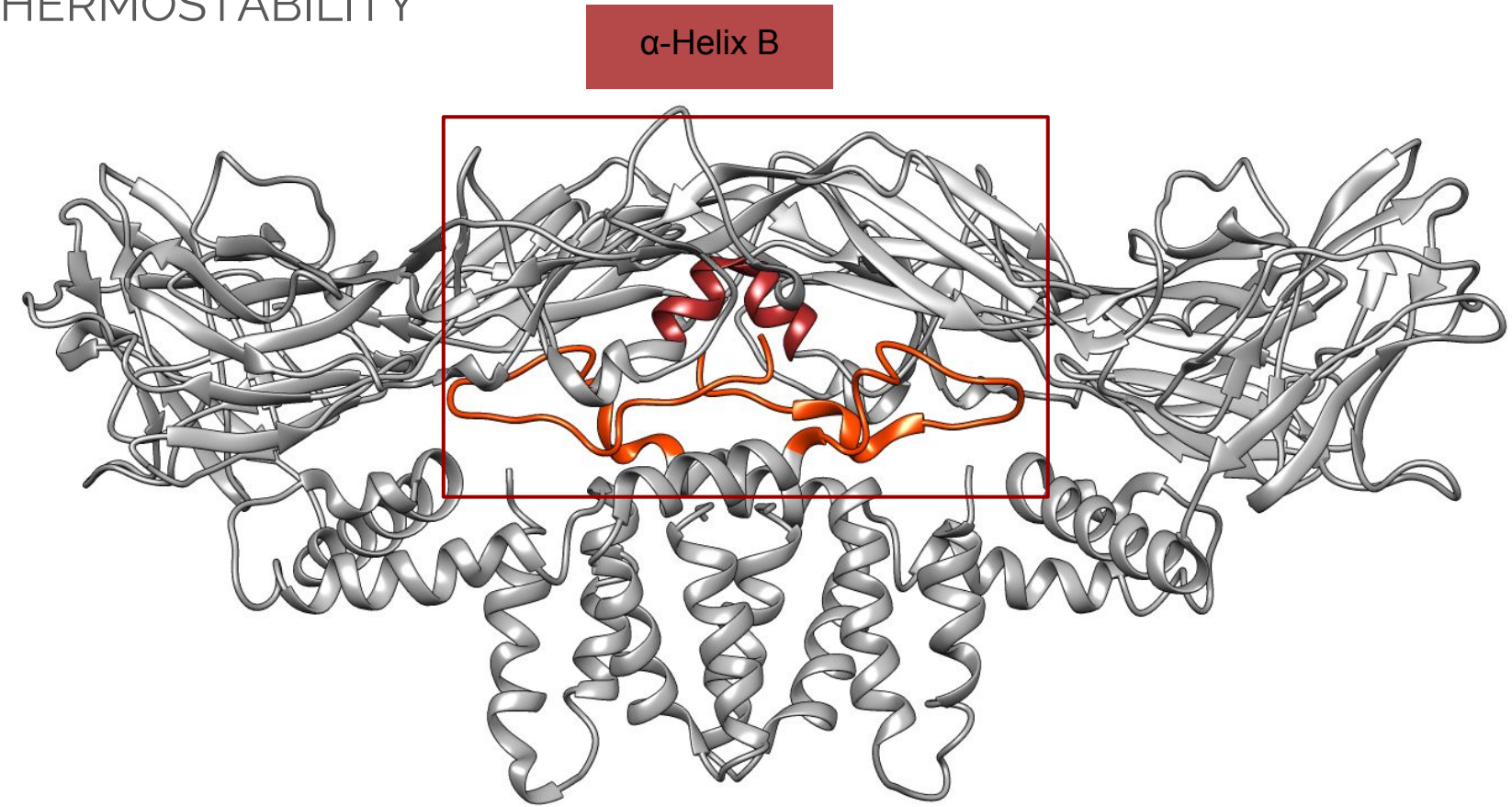


H-Bonds

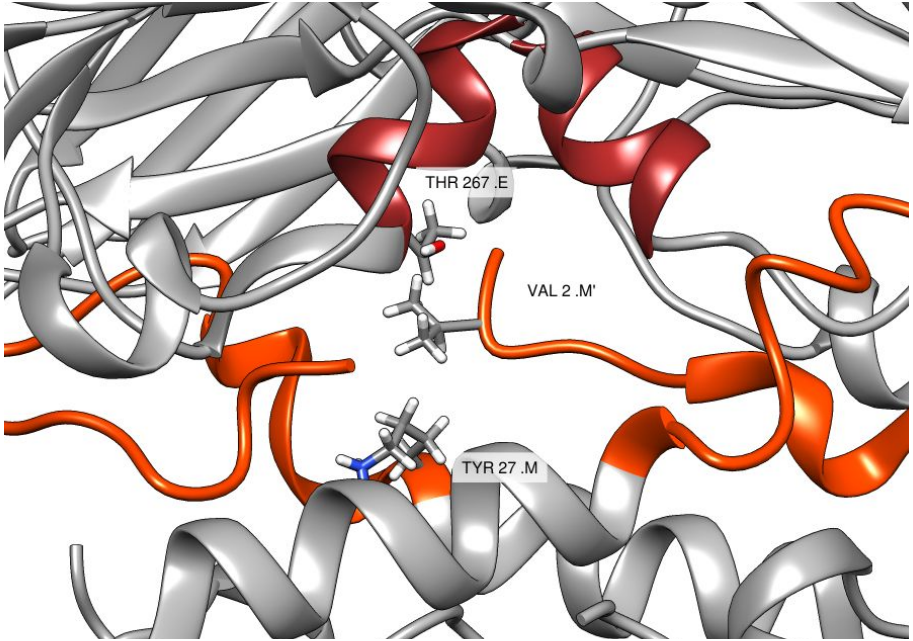


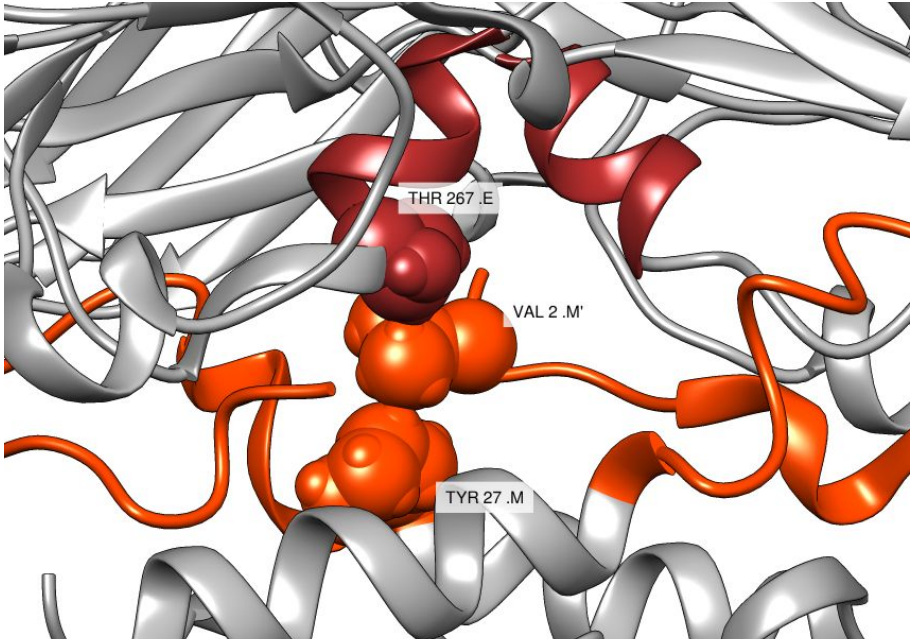
Salt-bridge

EM PROTEINS
THERMOSTABILITY



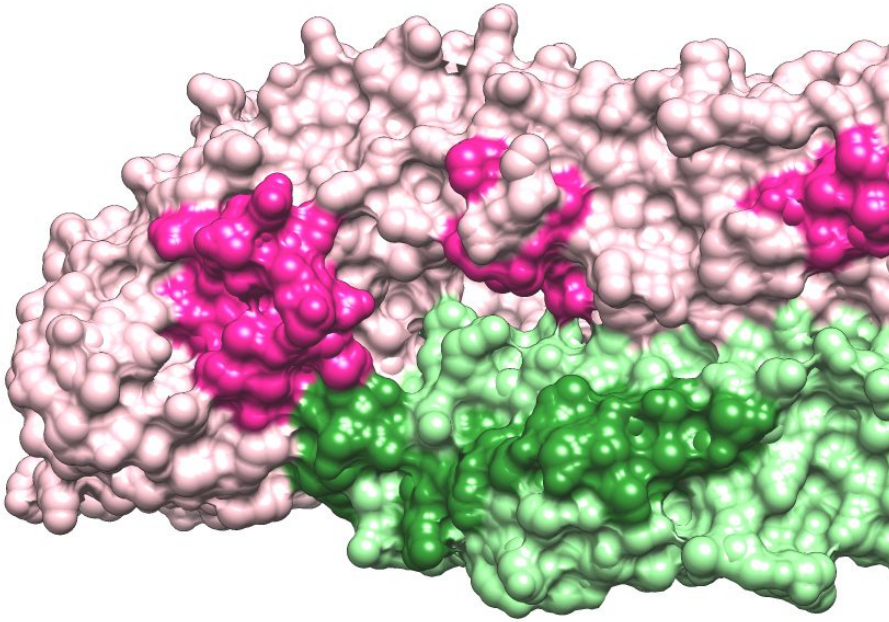
EM PROTEINS
THERMOSTABILITY





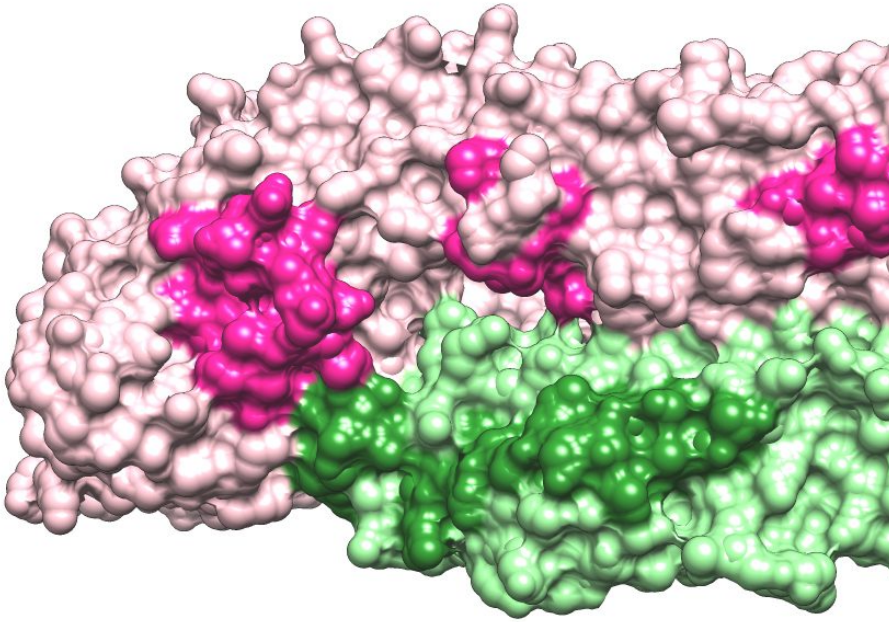
Immunogenicity

Antibodies

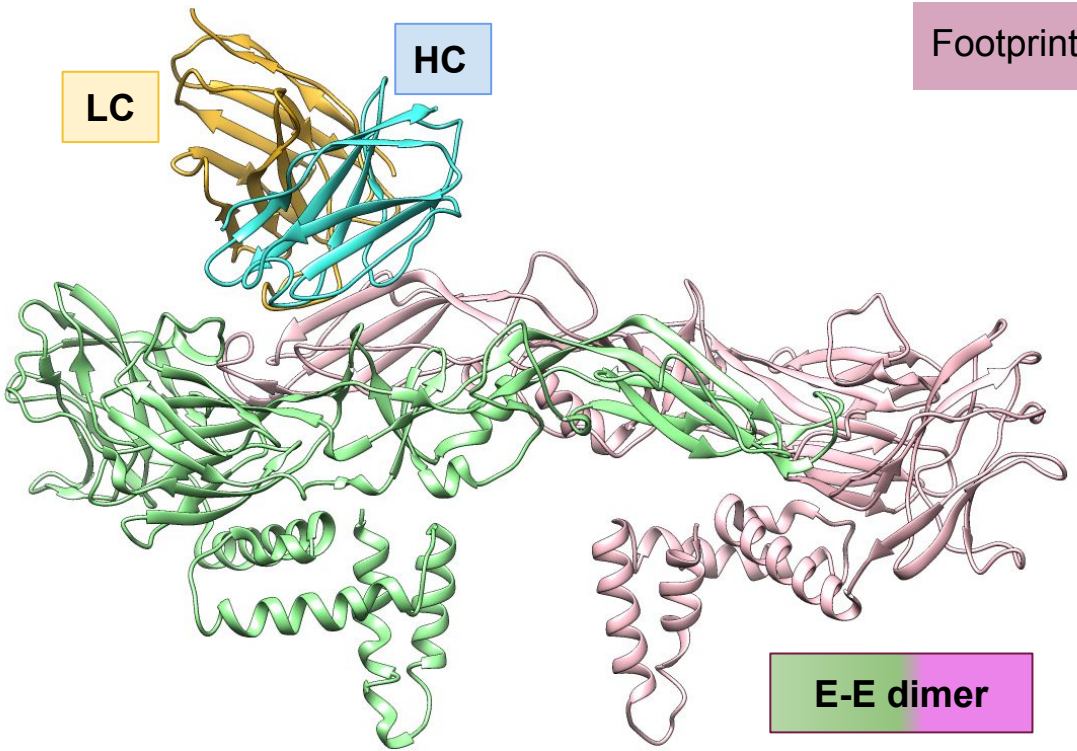


Immunogenicity

Antibodies: ZIKV-195



ZIKV-195: POTENT NEUTRALIZING HUMAN MONOCLONAL ANTIBODY

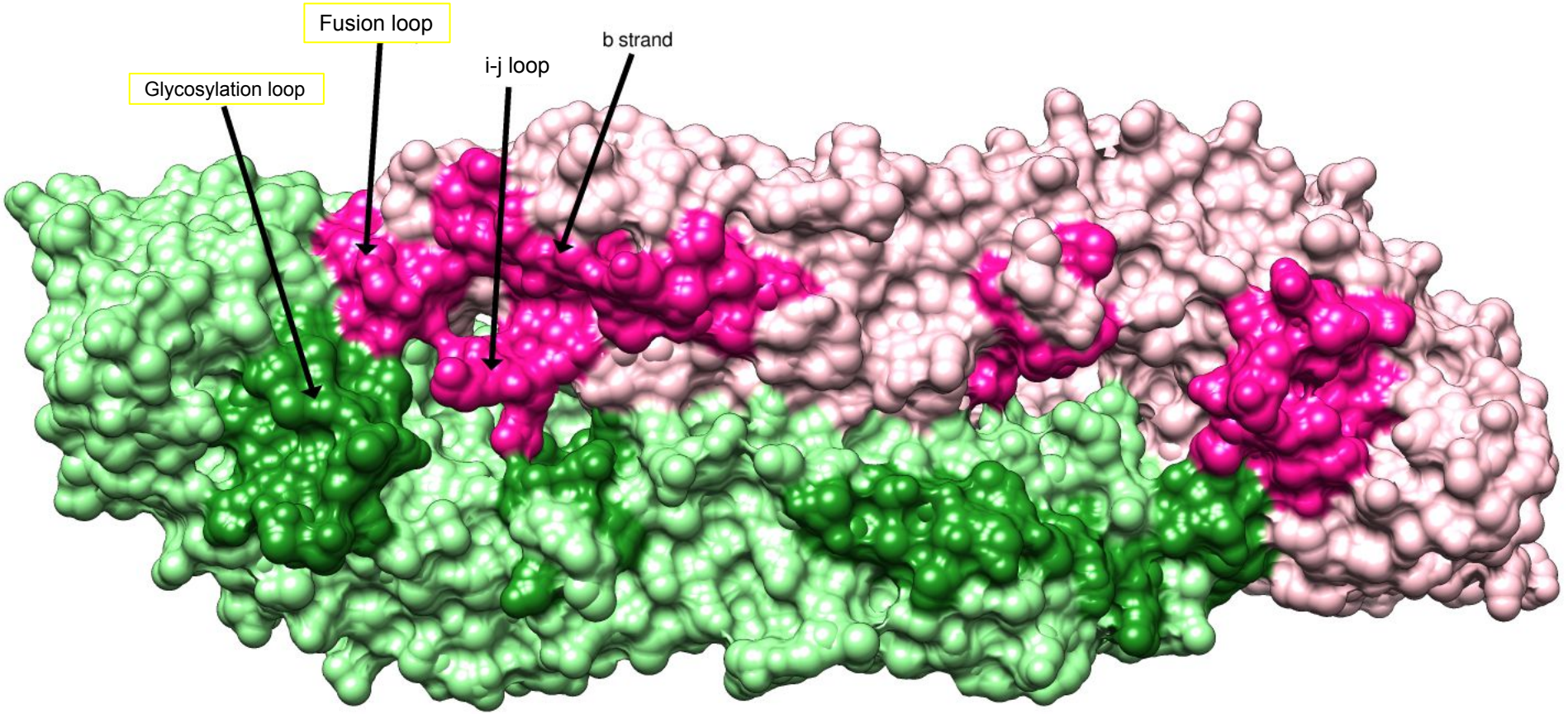


Footprint → Secondary structural elements of DI and DII

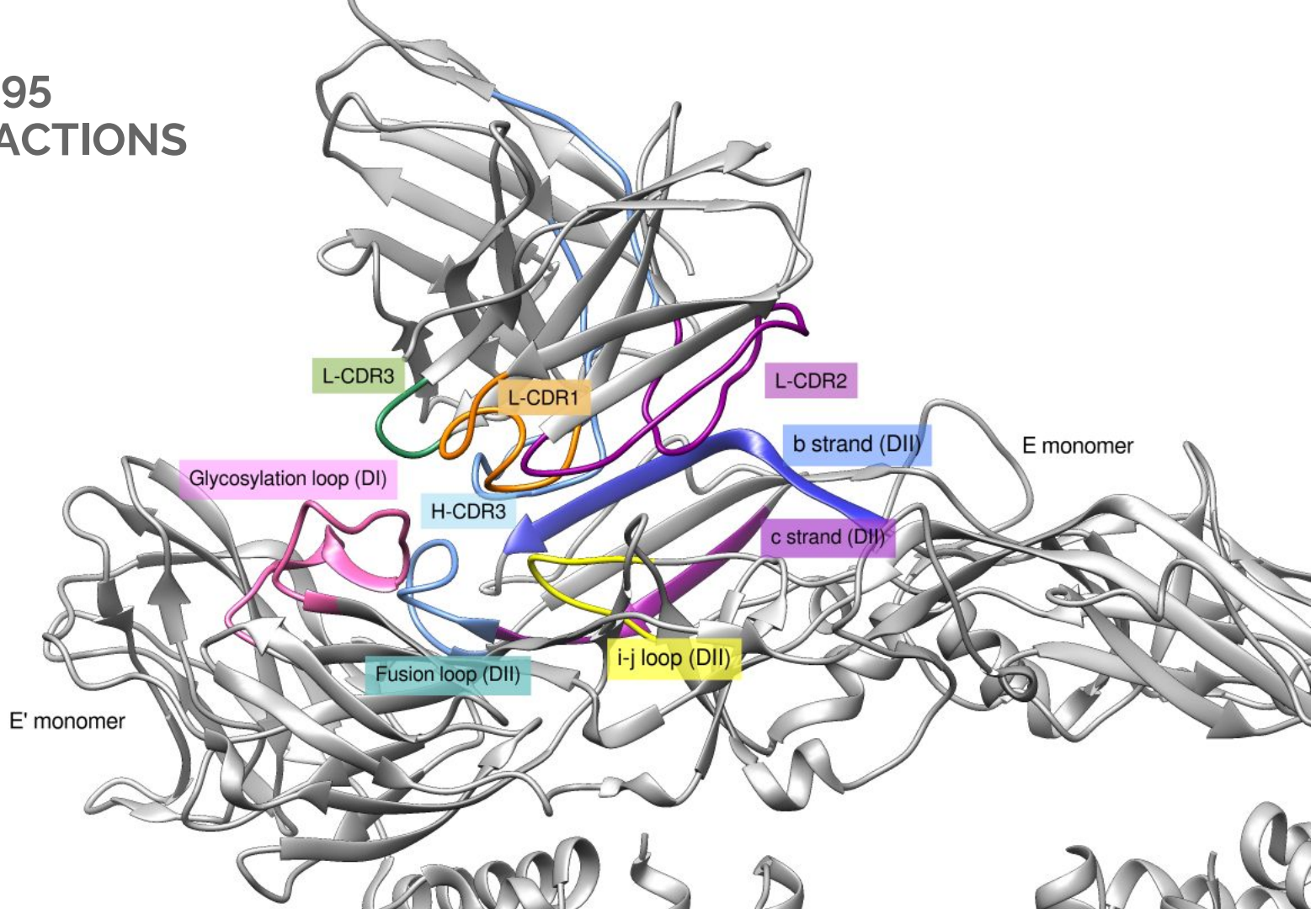
Binds to EE dimers

Specific for ZIKA virus

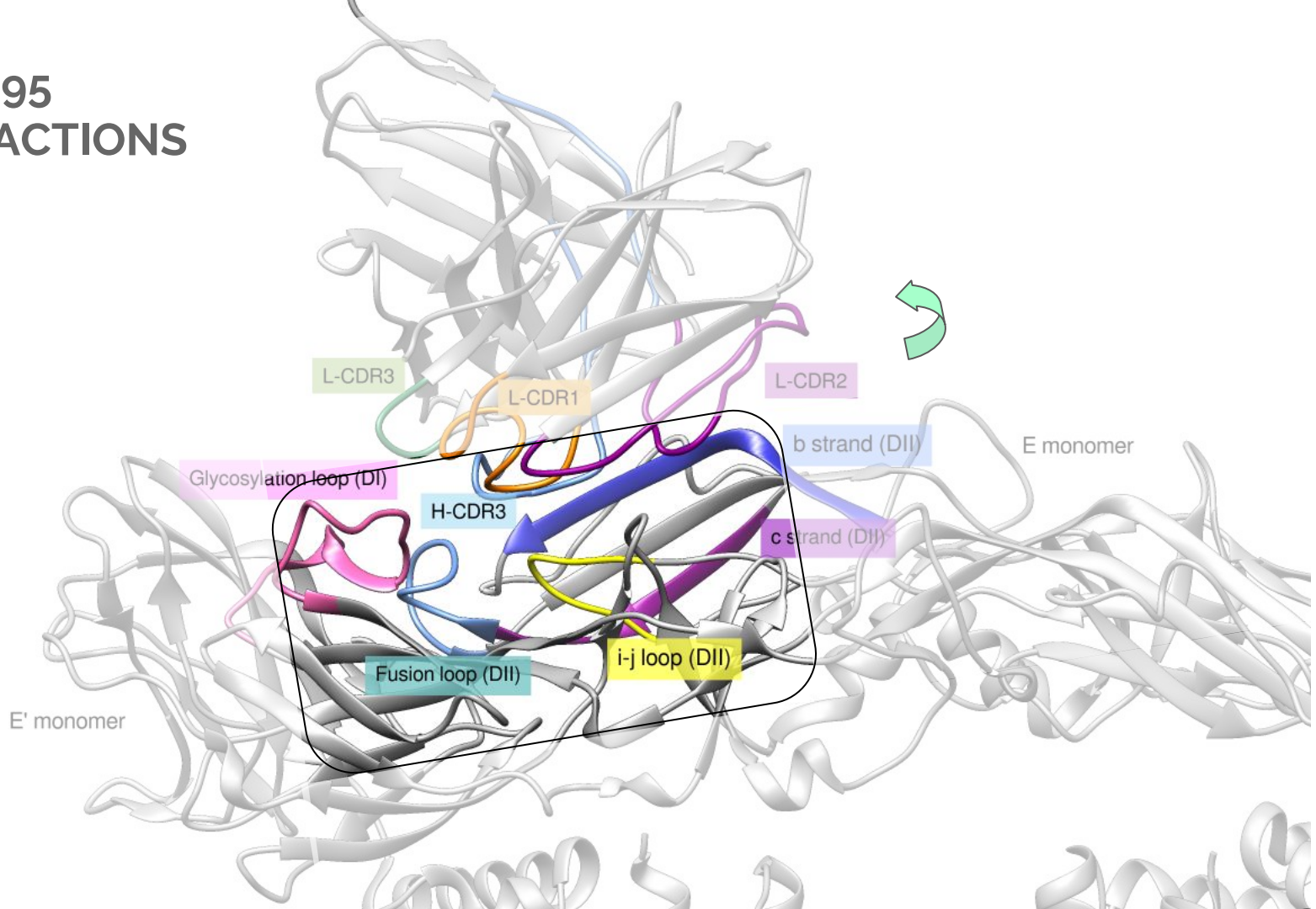
ZIKV-195 VIRAL PATCH



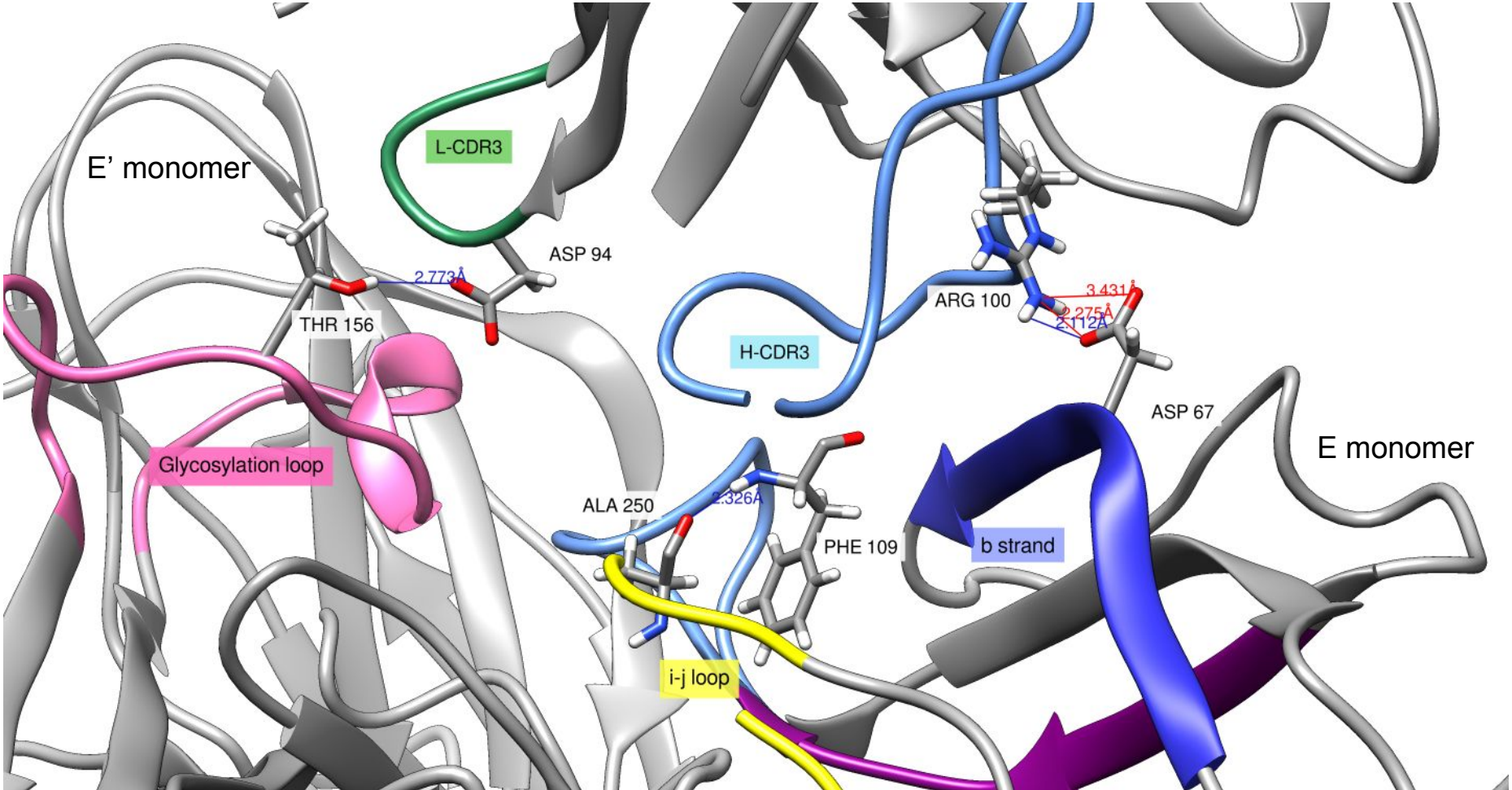
ZIKV-195 INTERACTIONS



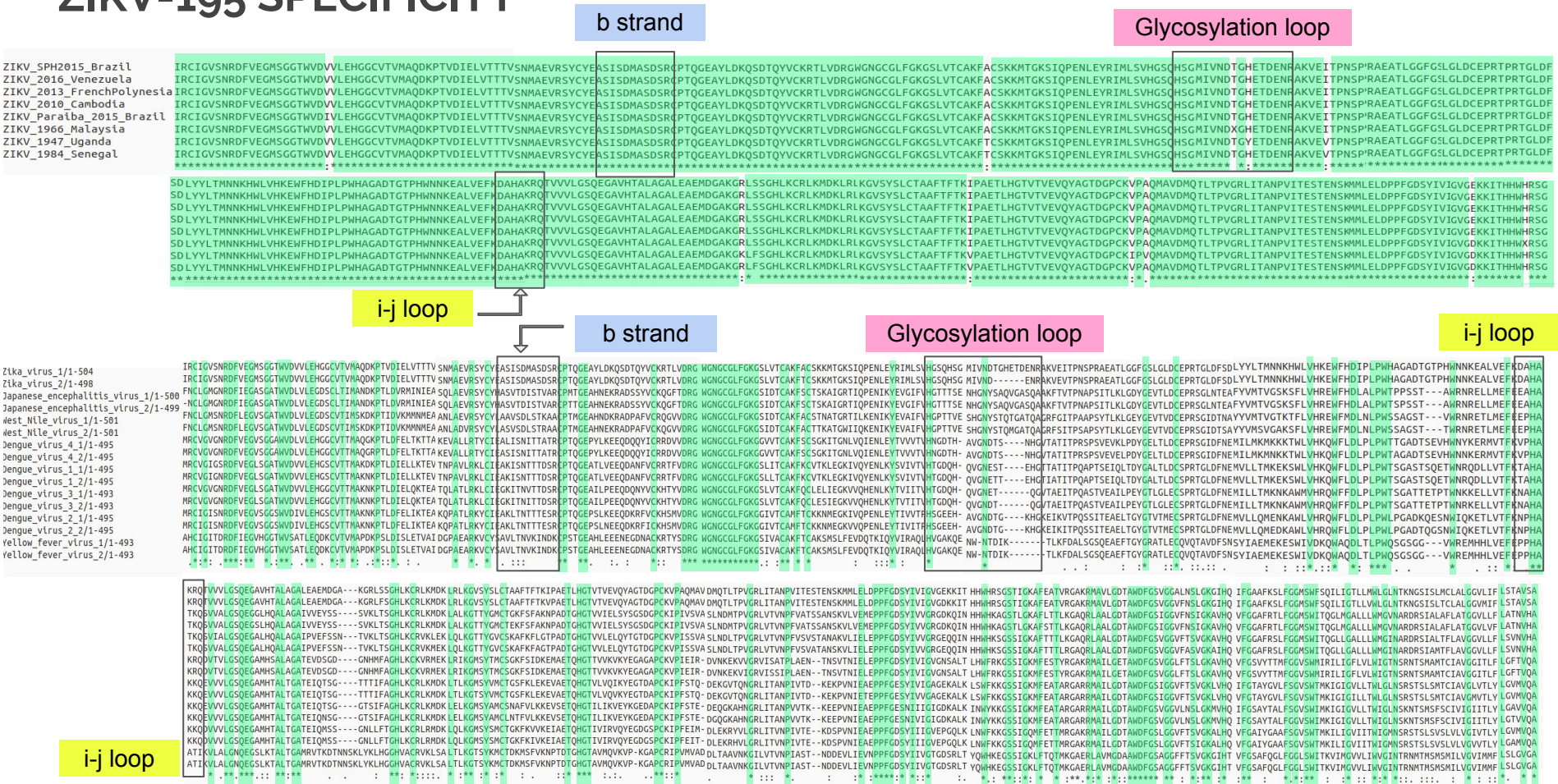
ZIKV-195 INTERACTIONS



ZIKV-195 INTERACTIONS

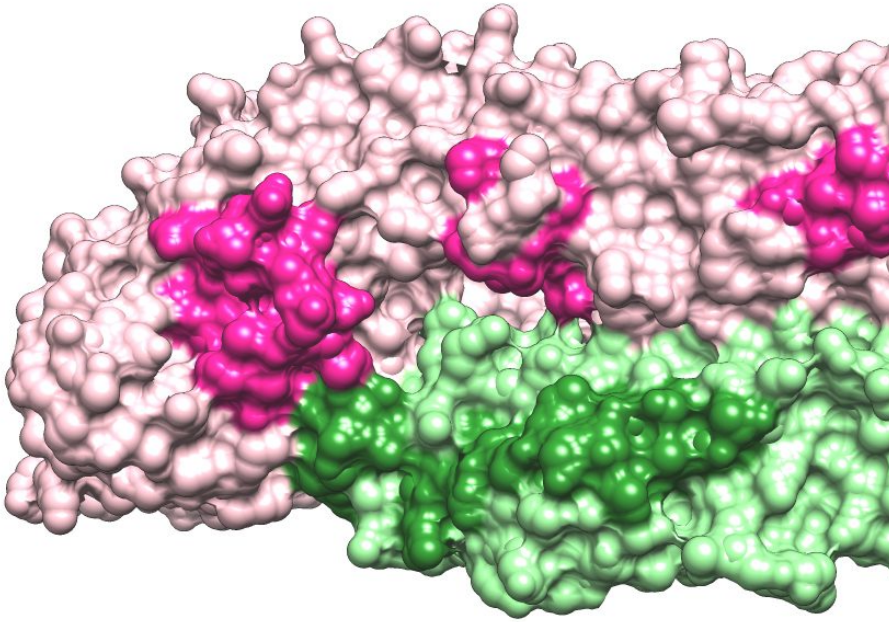


ZIKV-195 SPECIFICITY



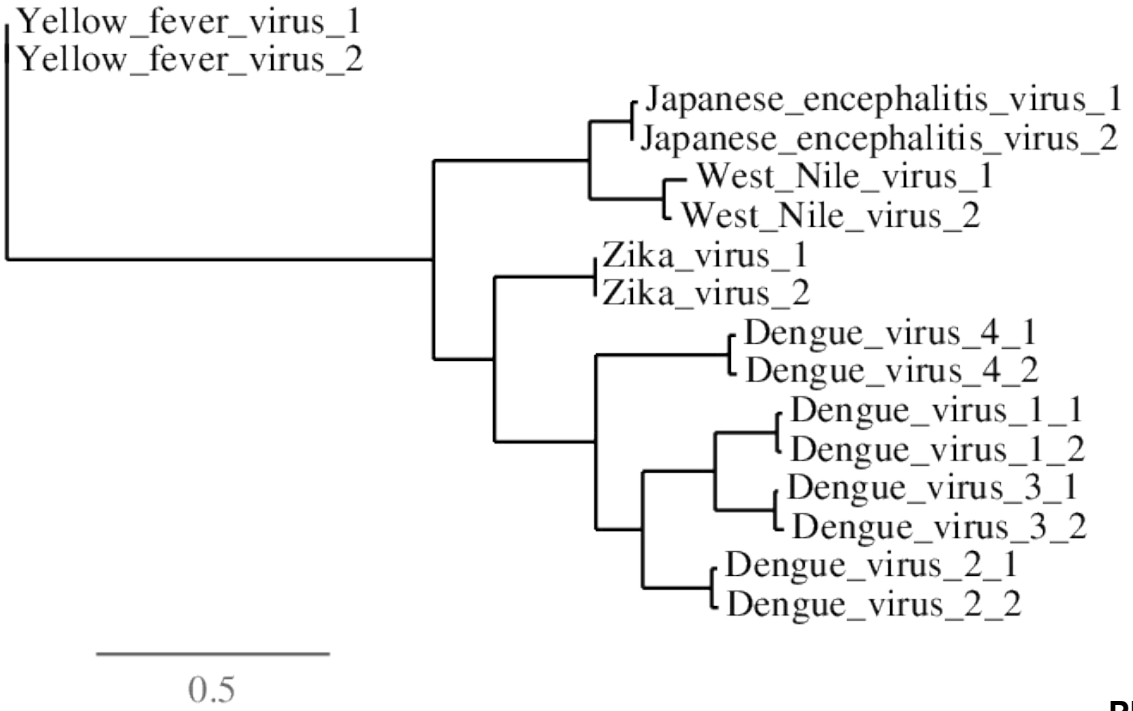
Immunogenicity

Antibodies: A-11



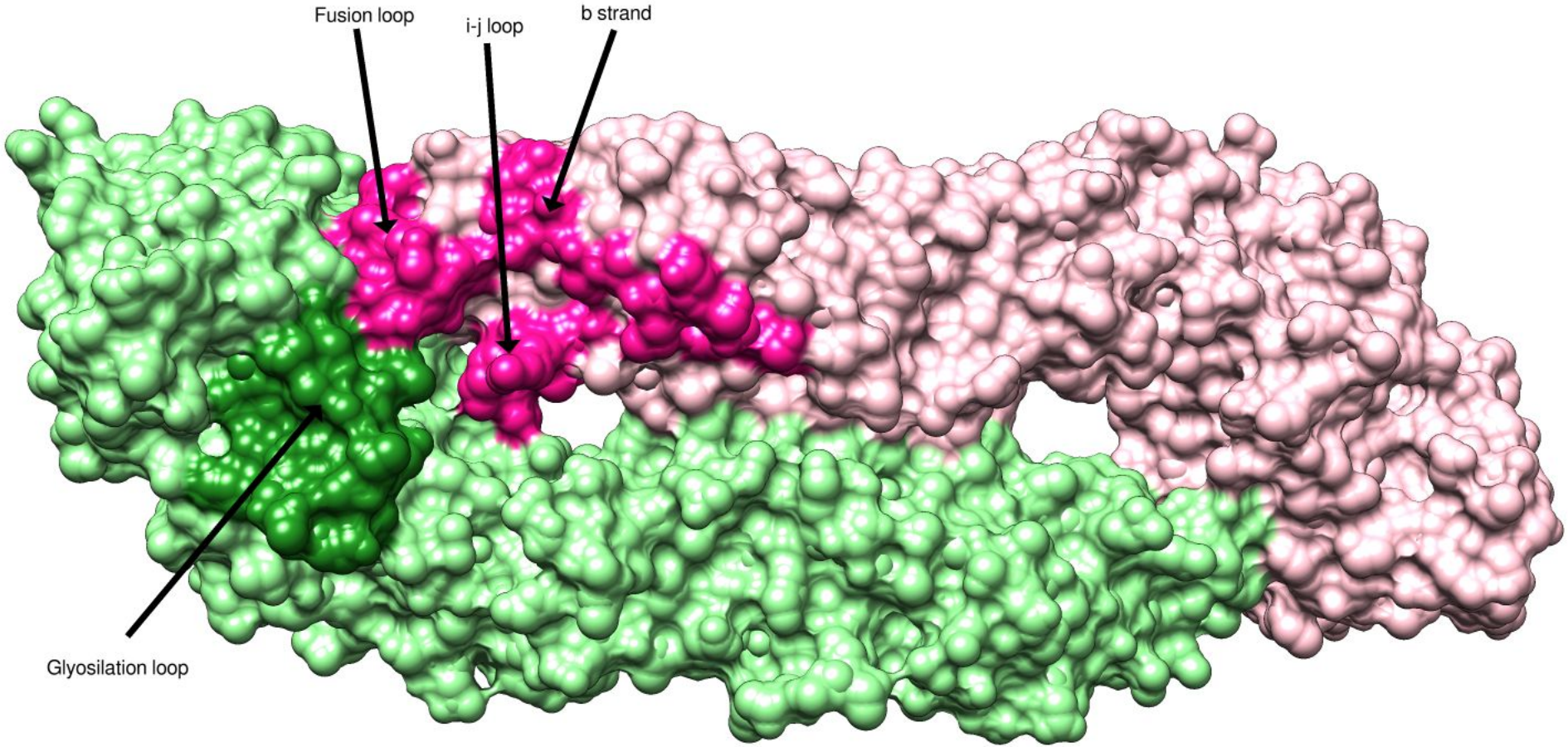
ANTIBODY CROSS-NEUTRALIZATION: A11

PHYLOGENETIC TREE

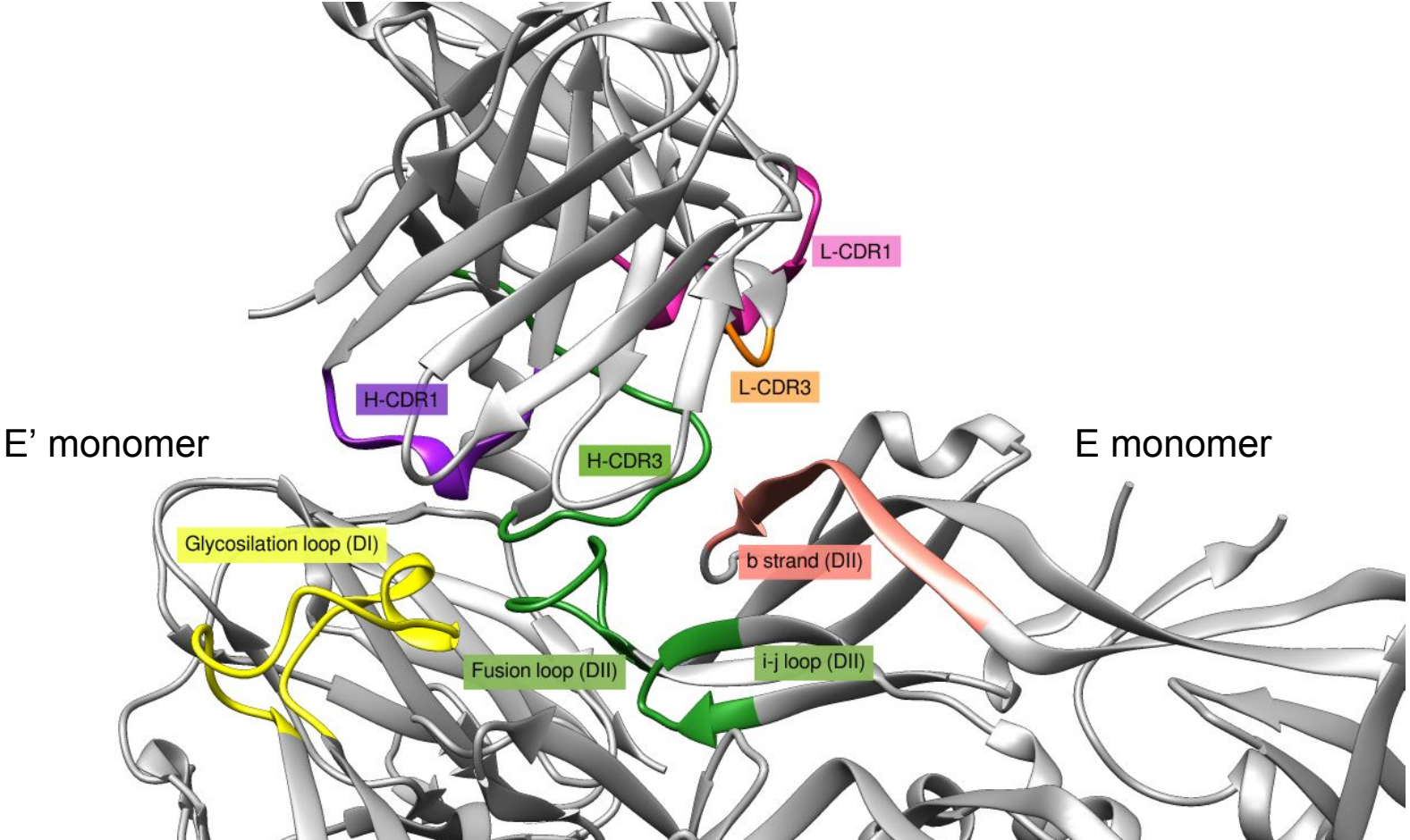


Phylogenetic tree made by phylogeny.fr

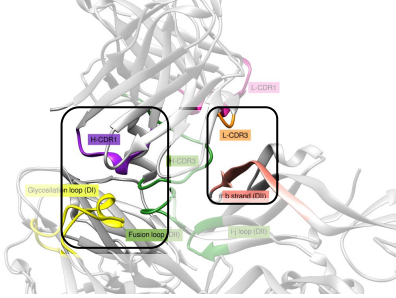
ANTIBODY CROSS-NEUTRALIZATION: A11 VIRAL PATCH



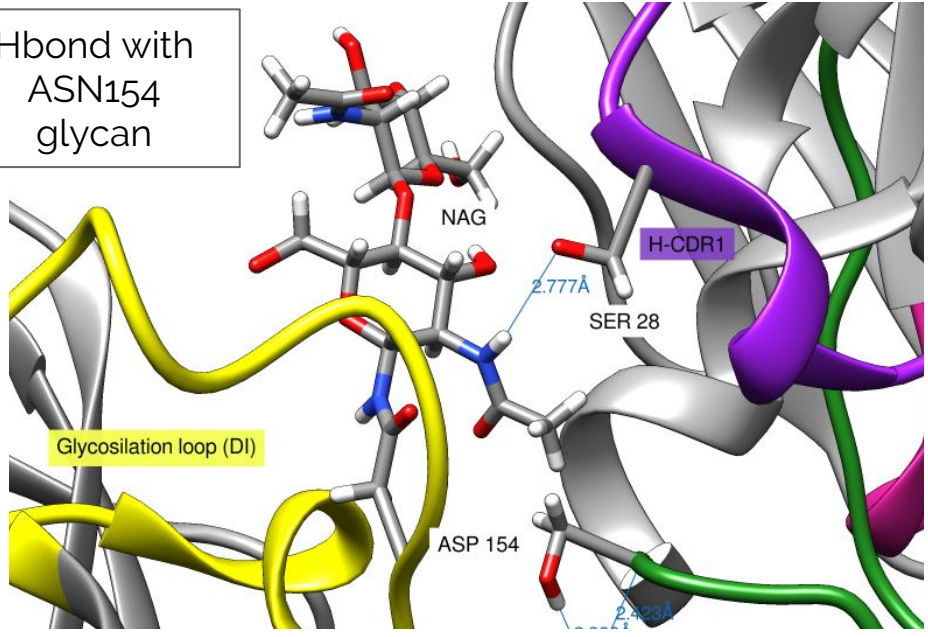
ANTIBODY CROSS-NEUTRALIZATION: A11 BINDING ZONES

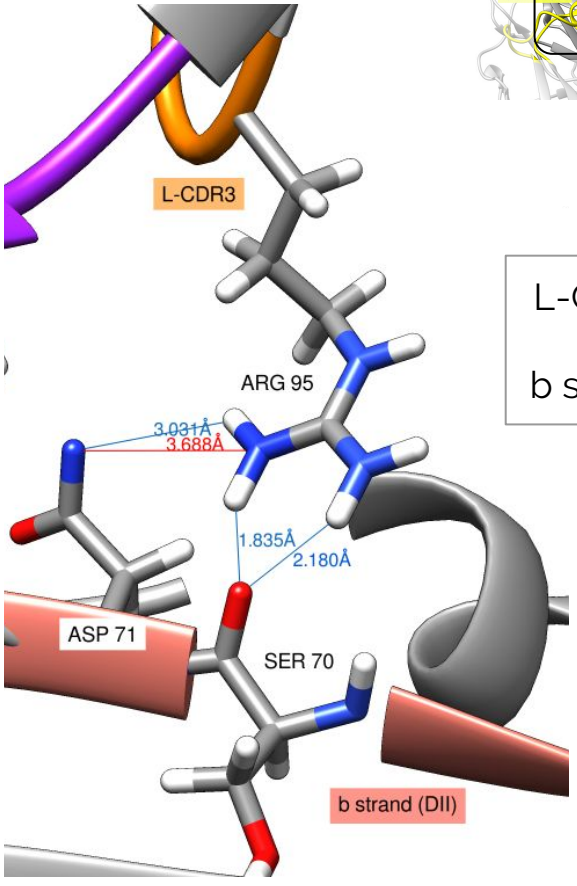


ANTIBODY CROSS-NEUTRALIZATION: A11 INTERACTIONS



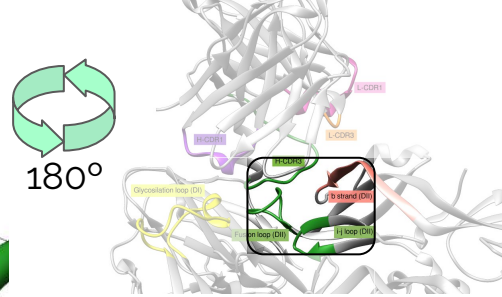
Hbond with ASN154 glycan

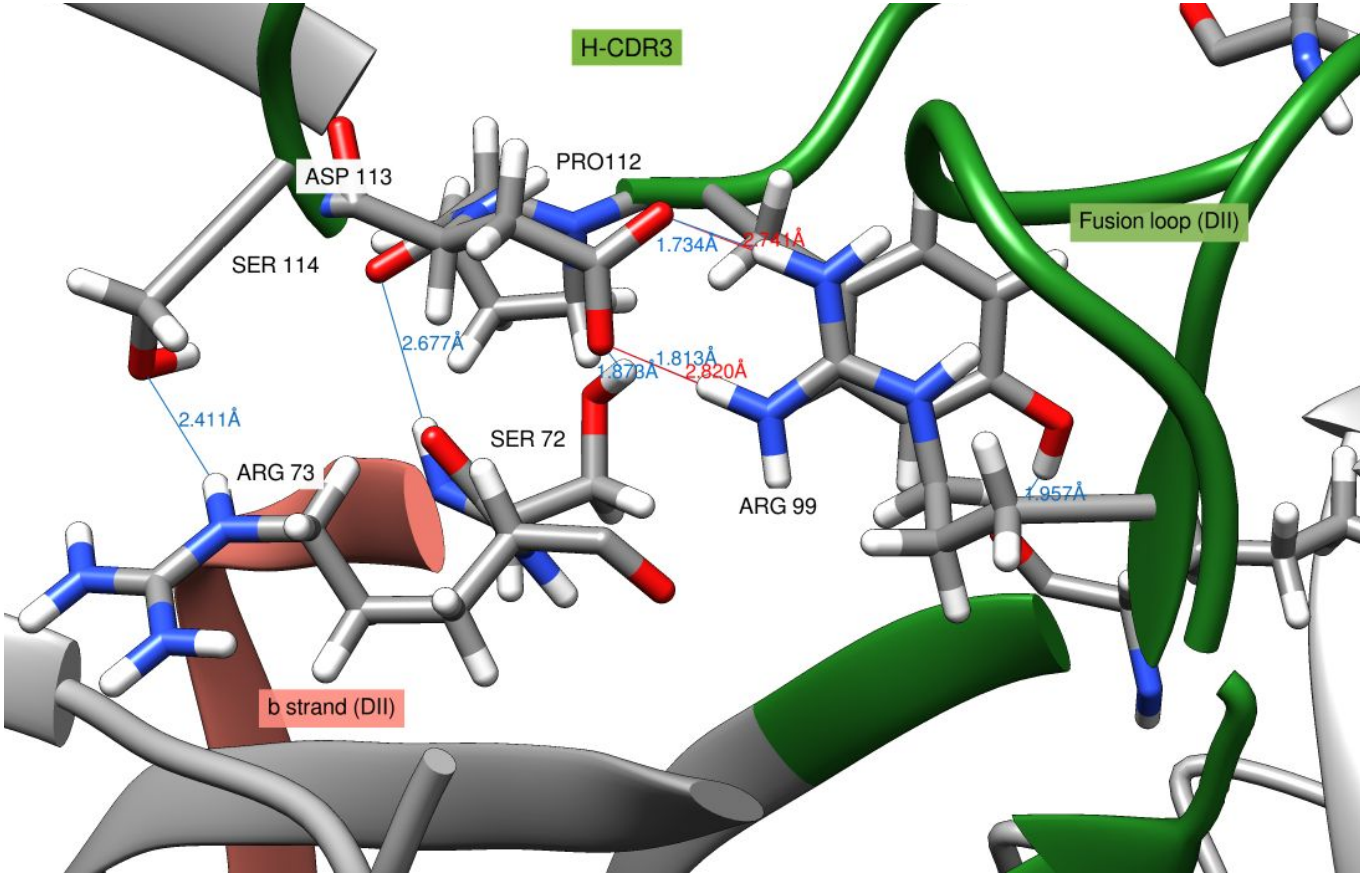




L-CDR3
+
b strand

ANTIBODY CROSS-NEUTRALIZATION: A11 INTERACTIONS

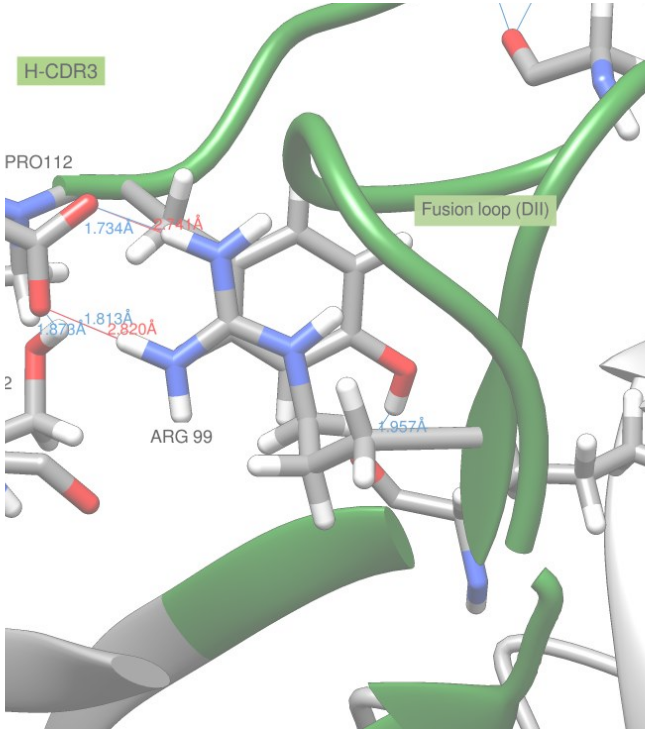




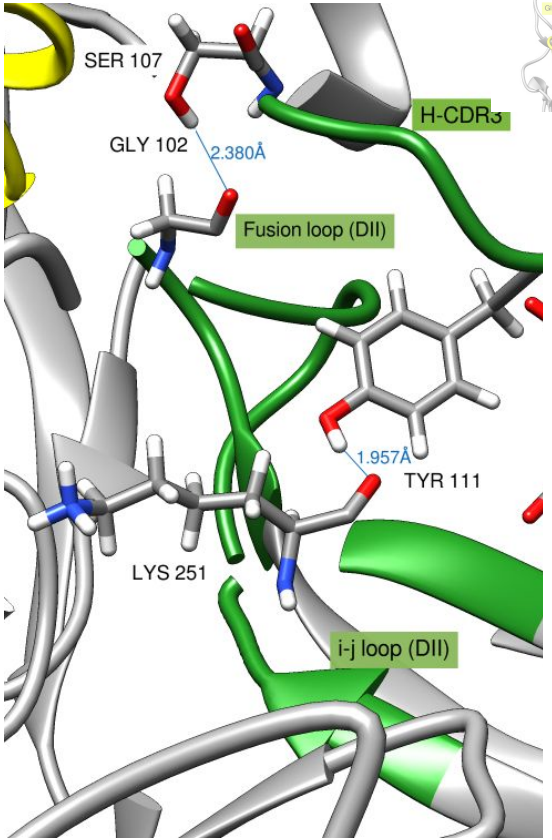
H-CDR3
+
b strand and Fusion loop

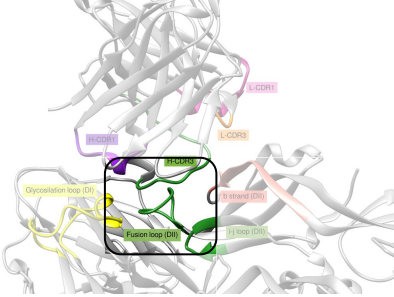
Back view

ANTIBODY CROSS-NEUTRALIZATION: A11 INTERACTIONS



180°

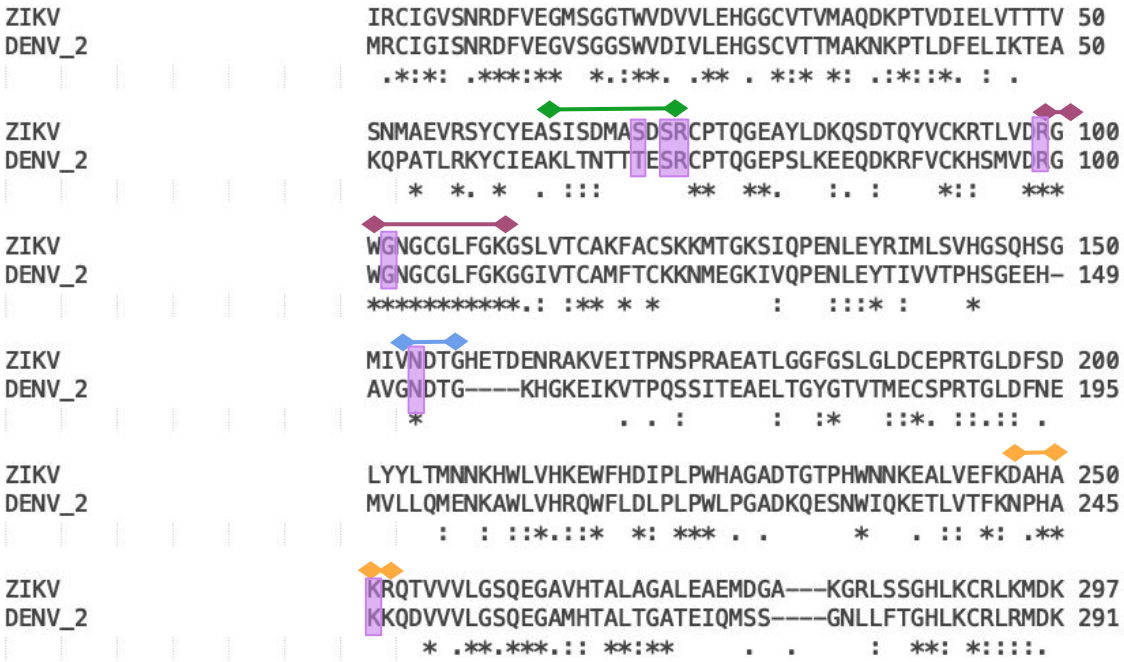




H-CDR3
+
Fusion loop and i-j loop

ANTIBODY CROSS-NEUTRALIZATION: A11

COMPARISON WITH DENV



b strand

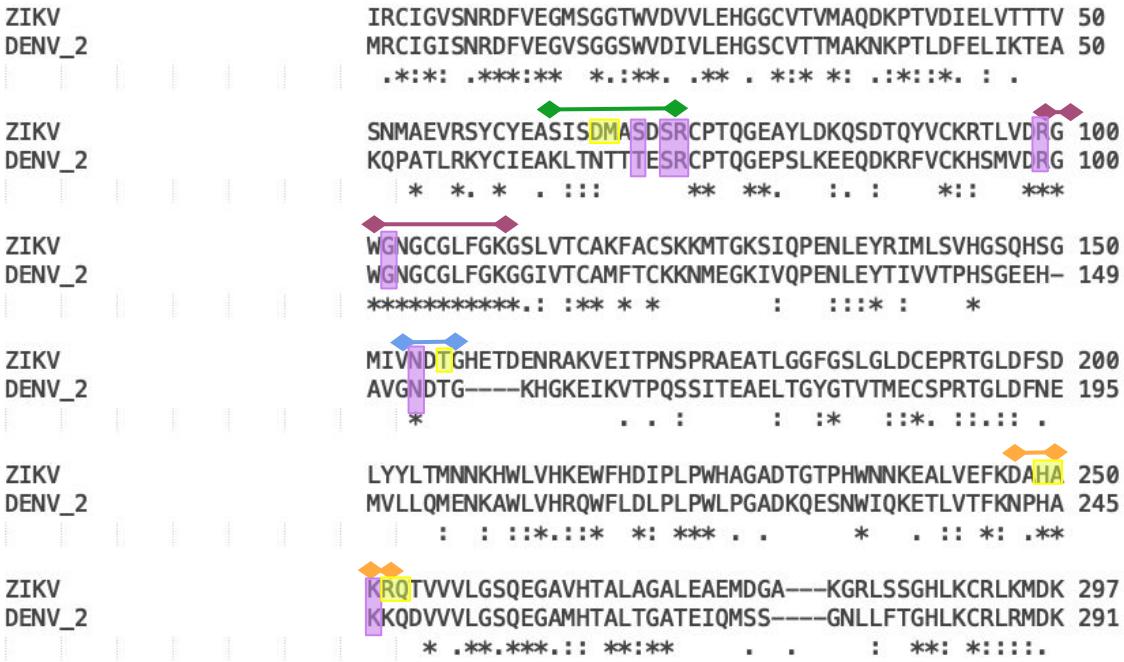
Fusion loop

Glycosylation loop

i-j loop

Cross-react binding residues

COMPARISON OF ZIKV-195 AND A11



b strand

Fusion loop

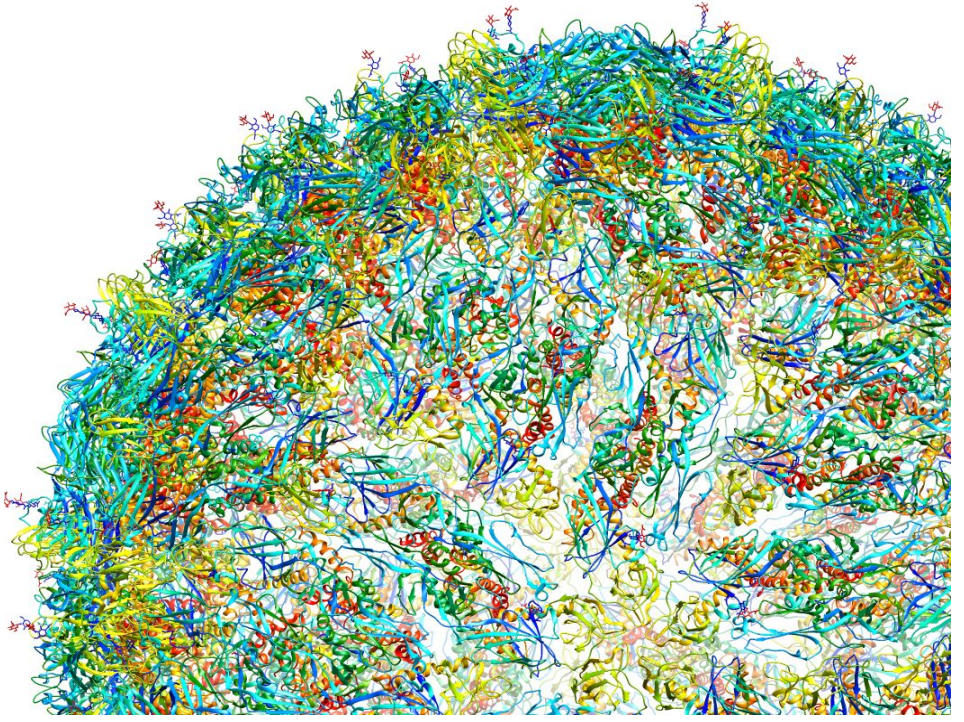
Glycosylation loop

i-j loop

Shared binding residues

Specific binding residues of ZIKV-195

Conclusions



TAKE-HOME MESSAGE

→ Simple sources can give rise to complex structures

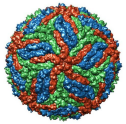
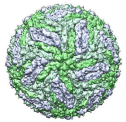
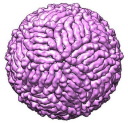
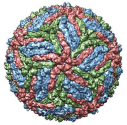
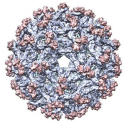
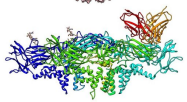
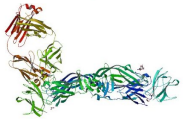
→ Viral variability can be useful to find therapeutic targets

→ Recent growth of interest in ZIKA virus

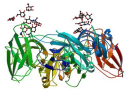
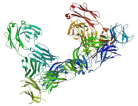
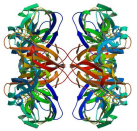
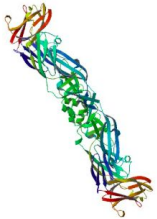
→ Refinement of structure resolving techniques are currently improving this study field

→ Zika virus is not as structurally studied as other Flaviviruses.

MATERIALS

	PDB code	Protein/Organism	Resolution	Method	Reference (DOI)
	6CO8	Proteins E and M (ZIKV)	3.1 Å	Electron Microscopy	10.1016/j.str.2018.05.006
	3J27	Proteins E and M (DENV2)	3.6 Å	Electron Microscopy	10.1038/nsmb.2463
	5IZ7	Thermally-stable ZIKV	3.7 Å	Electron Microscopy	10.1038/nature17994
	5IRE	Proteins E and M (ZIKV)	3.8 Å	Electron Microscopy	10.1126/science.aaf5316
	5U4W	Proteins E, M and pr domain (ZIKV and DENV2)	9.1 Å	Electron Microscopy	10.1038/nsmb.3352
	6MID	ZIKV-195 + E dimer	4 Å	Electron Microscopy	10.1073/pnas.1815432116
	5LCV	A11 Fab + E dimer	2.64 Å	X-Ray Diffraction	10.1038/nature18938

MATERIALS

	PDB code	Protein/Organism	Resolution	Method	Reference (DOI)
	1UZG	Proteins M and E (DENV3)	3.5 Å	X-Ray Diffraction	10.1128/JVI.79.2.1223-1231.2005
	3UAJ	Proteins M and E (DENV4)	3.2 Å	X-Ray Diffraction	10.1038/emboj.2011.439
	2HG0	Protein E (WNV)	3 Å	X-Ray Diffraction	10.1128/JVI.01125-06
	3P54	Protein E (JEV)	2.097 Å	X-Ray Diffraction	10.1128/JVI.06072-11
	6iW4	Protein E (YEV)	2,801 Å	X-Ray Diffraction	10.1016/j.celrep.2018.12.065

References

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10. Sevvana M, Long F, Miller A, Klose T, Buda G, Sun L et al. Refinement and Analysis of the Mature Zika Virus Cryo-EM Structure at 3.1 Å Resol. Struct. 2018;26(9):1169-1177.
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13. Zhang X, Sheng J, Austin SK, Hoornweg TE, Smit JM, Kuhn RJ et al. Structure of Acidic pH Dengue Virus Showing the Fusogenic Glycoprotein Trimers. J Virol. 2015;89(1): 743–750.

Questions

- Which is the **most used** technique to solve viral structures?
  a) X-ray diffraction
  b) Cryo-electromicroscopy
  c) NMR
  d) a and b are correct
  e) all of them are correct

- Which of the following sentences is correct:
  a) The Zika virus envelope symmetry consists of one axis of each fold (5, 3 and 2).
  b) The Zika virus symmetry is icosahedral.
  c) Not all the Flaviviruses share the same symmetry.
  d) The Zika virus has a T=4-like organization of surface.
  e) The Zika virus envelope is composed by 200 asymmetric units.

- The asymmetric unit of Zika virus envelope is composed by:
  a) 60 trimers of E proteins
  b) The E-M heterodimers trimer
  c) A raft of three E homodimers
  d) 5-fold axis
  e) None of them are correct

- Which of the following sentences is false:
  a) The envelope protein E plays an important role in cell attachment but not in antigenicity.
  b) The envelope protein E is formed by three ectodomains (DI, DII, DIII).
  c) DII and DIII contains the neutralizing epitopes.
  d) Domains DII and DIII belong to alpha-beta class (CATH classification).
  e) The envelope protein E is structurally similar among Flaviviruses.

Questions

- About protein E loops, choose the correct answer:
  a) There are no variable loop in E protein.
  b) Zika virus has 10 loops functionally relevant.
  c) The fusion loop is the most conserved loop of E protein.
  d) The DI-DIII hinge is responsible for the viral interaction with the cellular receptor.
  e) All of them are correct.

- Related to the Zika virus surface:
  a) Needs an aqueous environment to survive, that's why the surface of E protein is mainly polar.
  b) E protein is also anchored on the membrane, so the transmembrane domain has a hydrophobic surface.
  c) a and b are correct.
  d) Even though the surface of the ectodomains is polar, there is a hydrophobic pocket.
  e) All of them are correct.

- The interactions between E monomers:
  a) Are mainly polar.
  b) Depends on the symmetry axis they adjacent to.
  c) A and B are correct.
  d) Determine the interaction of the M protein with the pr peptide.
  e) All of the above are correct.

- M protein:
  a) Is bigger than E protein.
  b) Is mainly formed by beta strands.
  c) In the mature form of the Zika virus is exposed to the solvent.
  d) Is formed by three alpha helices (H1, H2 and H3)
  e) There are no hydrophobic interactions between E and M proteins.

Questions

- Which of the following sentences is false:
  - **a) T cell epitopes are mainly located in E protein transmembrane domain.**
  - b) ZIKV-195 is a potent human monoclonal antibody that prevents the formation of fusogenic trimers.
  - c) There is cross-neutralization between flavivirus, mainly between Zika and Dengue.
  - d) Some of the Zika virus epitopes are negatively selected.
  - e) The specificity of the antibody ZIKV-195 for the Zika virus is due to the conservation of certain residues among ZIKA virus strains.
- Select the correct answer:

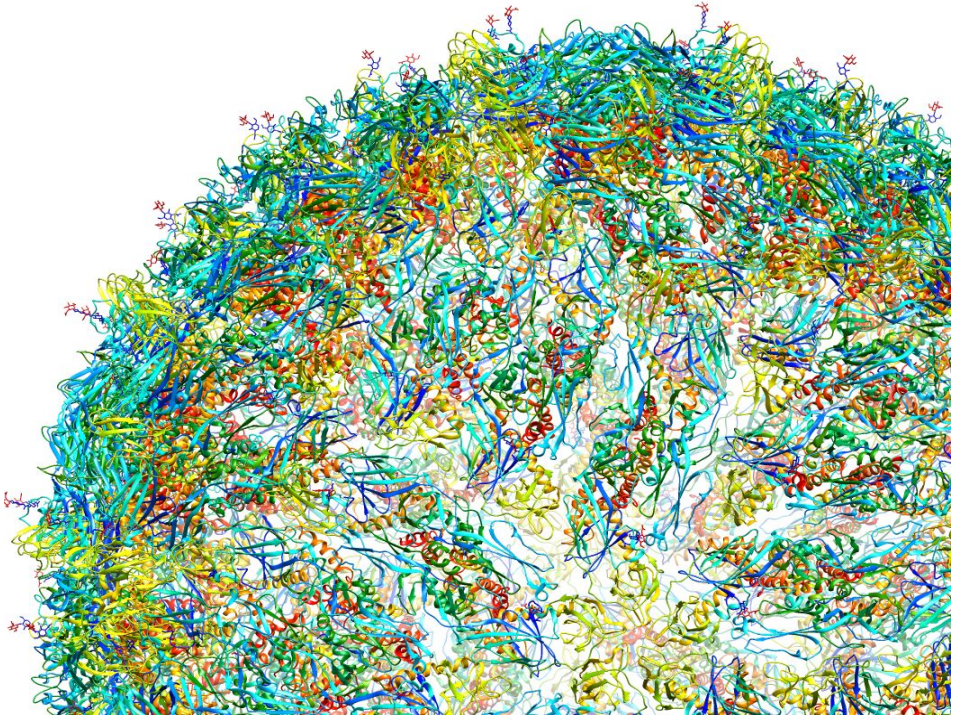
1. The k-l Loop of DENV has been associated to the change of conformation of the hydrophobic pocket.
2. The Histidine 323 is thought to be the molecular sensor for triggering fusion.
3. The hydrophobic pocket is hiding the fusion loop in neutral pH.
4. The hydrophobic is hiding the fusion loop in all of the viral conformations.
   - a) 1, 2 and 3
   - b) 1 and 3
   - c) 2 and 4
   - d) 4
   - e) 1, 2, 3 and 4

Thank you for your attention

Campos M, Canales A,
Díaz J, Llerena M.

Structural Biology
Human Biology, UPF
2018-2019

Annexes



Flavivirus glycoprotein, central and dimerisation domains

ANNEX

HMM alignment

```
Zika_virus_1                        IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTV
Zika_virus_2                        IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTV
Zika_virus_3                        IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTV
Zika_virus_4                        IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTV
Dengue_virus_1_1                    MRCVGIGSRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTEV
Dengue_virus_1_2                    MRCVGIGNRDFVEGLSGATWVDVVLEHGSCVTTMAKNKPTLDIELLKTEV
Dengue_virus_1_3                    MRCVGIGSRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTEV
Dengue_virus_1_4                    MRCVGIGSRDFVEGLSGATWVDVVLEHGSCVTTMAKNKPTLDIELLKTEV
Dengue_virus_2_1                    MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEA
Dengue_virus_2_2                    MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEA
Dengue_virus_2_3                    MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEA
Dengue_virus_2_4                    MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEA
Dengue_virus_3_1                    MRCVGVGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELQKTEA
Dengue_virus_3_2                    MRCVGVGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELQKTEA
Dengue_virus_3_3                    -RCVGVGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELQKTEA
Dengue_virus_3_4                    MRCVGVGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELQKTEA
Dengue_virus_4_1                    MRCVGVGNRDFVEGVSGGAWVDLVLEHGGCVTTMAQGKPTLDFELTKTTA
Dengue_virus_4_2                    MRCVGVGNRDFVEGVSGGAWVDLVLEHGGCVTTMAQGRPTLDFELTKTTA
Dengue_virus_4_3                    MRCVGVGNRDFVEGVSGGAWVDLVLEHGGCVTTMAQGKPTLDFELTKTTA
Dengue_virus_4_4                    MRCVGVGNRDFVEGVSGGAWVDLVLEHGGCVTTMAQGKPTLDFELTKTTA
Japanese_encephalitis_virus_1       FNCLGMGNRDFIEGASGATWVDLVLEGDSCLTIMANDKPTLDVRMINIEA
Japanese_encephalitis_virus_2       -NCLGMGNRDFIEGASGATWVDLVLEGDSCLTIMANDKPTLDVRMINIEA
Japanese_encephalitis_virus_3       ----------EGASGATWVDLVLEGDSCLTIMANDKPTLDVRMINIEA
Japanese_encephalitis_virus_4       ----------EGASGATWVDLVLEGDSCLTILANDKPTLDVRMINIEA
West_Nile_virus_1                   FNCLGMSNRDFLEGVSGATWVDLVLEGDSCVTIMSKDKPTIDVKMMNMEA
West_Nile_virus_2                   FNCLGMSNRDFLEGVSGATWVDLVLEGDSCVTIMSKDKPTIDVKMMNMEA
West_Nile_virus_3                   FNCLGMSNRDFLEGVSGATWVDLVLEGDSCVTIMSKDKPTIDVKMMNMEA
West_Nile_virus_4                   FNCLGMSNRDFLEGVSGATWVDLVLEGDSCVTIMSKDKPTIDVKMMNMEA
Yellow_fever_virus_1                AHCIGITDRDFIEGVHGGTWVSATLEQDKCVTVMAPDKPSLDISLETVAI
Yellow_fever_virus_2                AHCIGITDRDFIEGVHGGTWVSATLEQDKCVTVMAPDKPSLDISLETVAI
Yellow_fever_virus_3                AHCIGITDRDFIEGVHGGTWVSATLEQDKCVTVMAPDKPSLDISLETVAI
Yellow_fever_virus_4                AHCIGITDRDFIEGVHGGTWVSATLEQDKCVTVMAPDKPSLDISLETVAI
#=GC RF                             xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
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Zika_virus_1                        SNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRG
Zika_virus_2                        SNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRG
Zika_virus_3                        SNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRG
Zika_virus_4                        SNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRG
Dengue_virus_1_1                    TNPAVLRKLCIEAKISNTTTDSRCPTQGEATLVEEQDANFVCRRTFVDRG
Dengue_virus_1_2                    TNPAVLRKLCIEAKISNTTTDSRCPTQGEATLVEEQDANFVCRRTFVDRG
Dengue_virus_1_3                    TNPAILRKLCIEAKISNTTTDSRCPTQGEATLVEEQDANFVCRRTFVDRG
Dengue_virus_1_4                    TNPAVLRKLCIEAKISNTTTDSRCPTQGEATLVEEQDANFVCRRTFVDRG
Dengue_virus_2_1                    KQPATLRKYCIEAKLTNTTTESRCPTQGEPSLKEEQDKRFVCKHSMVDRG
Dengue_virus_2_2                    KQPATLRKYCIEAKLTNTTTESRCPTQGEPSLNEEQDKRFICKHSMVDRG
Dengue_virus_2_3                    KQPATLRKYCIEAKLTNTTTESRCPTQGEPSLNEEQDKRFICKHSMVDRG
Dengue_virus_2_4                    KQPATLRKYCIEAKLTNTTTESRCPTQGEPSLNEEQDKRFICKHSMVDRG
Dengue_virus_3_1                    TQLATLRKLCIEGKITNVTTDSRCPTQGEAILPEEQDQNYVCKHTYVDRG
Dengue_virus_3_2                    TQLATLRKLCIEGKITNITTDSRCPTQGEAILPEEQDQNYVCKHTYVDRG
Dengue_virus_3_3                    TQLATLRKLCIEGKITNITTDSRCPTQGEAVLPEEQDQNYVCKHTYVDRG
Dengue_virus_3_4                    TQLATLRKLCIEGKITNVTTDSRCPTQGEAILPEEQDQNYVCKHTYVDRG
Dengue_virus_4_1                    KEVALLRTYCIEALISNITTATRCPTQGEPYLKEEQDQQYICRRDVVDRG
Dengue_virus_4_2                    KEVALLRTYCIEASISNITTATRCPTQGEPYLKEEQDQQYICRRDVVDRG
Dengue_virus_4_3                    KEVALLRTYCIEASISNITTATRCPTQGEPYLKEEQDQQYICRRDVVDRG
Dengue_virus_4_4                    KEVALLRTYCIEASISNITTATRCPTQGEPYLKEEQDQQYICRRGVVDRG
Japanese_encephalitis_virus_1       SQLAEVRSYCYHASVTDISTVARCPMTGEAHNEKRADSSYVCKQGFTDRG
Japanese_encephalitis_virus_2       SQLAEVRSYCYHASVTDISTVARCPTTGEAHNEKRADSSYVCKQGFTDRG
Japanese_encephalitis_virus_3       SQLAEVRSYCYHASVTDISTVARCPTTGEAHNEKRADSSYVCKQGFTDRG
Japanese_encephalitis_virus_4       SQLAEVRSYCYHASVTDISTVARCPTTGEAHNEKRADSSYVCKQGFTDRG
West_Nile_virus_1                   ANLAEVRSYCYLAAVSDLSTKAACPTMGEAHNDKRADPAFVCRQGVVDRG
West_Nile_virus_2                   ANLADVRSYCYLASVSDLSTRAACPTMGEAHNEKRADPAFVCKQGVVDRG
West_Nile_virus_3                   ANLAEVRSYCYLATVSDLSTKAACPTMGEAHNDKRADPAFVCRQGVVDRG
West_Nile_virus_4                   ANLAEVRSYCYLATVSDLSTKAACPTMGEAHNDKRADPAFVCRQGVVDRG
Yellow_fever_virus_1                DGPAEARKVCYSAVLTNVKINDKCPSTGEAHLEEENEGDNACKRTYSDRG
Yellow_fever_virus_2                DGPAEARKVCYSAVLTNVKINDKCPSTGEAHLEEENEGDNACKRTYSDRG
Yellow_fever_virus_3                DGPAEARKVCYSAVLTNVKINDKCPSTGEAHLEEENEGDNACKRTYSDRG
Yellow_fever_virus_4                DGPAEARKVCYSAVLTNVKINDKCPSTGEAHLEEENEGDNACKRTYSDRG
#=GC RF                             xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
```

Flavivirus glycoprotein, central and dimerisation domains

ANNEX

HMM alignment

```
Zika_virus_1                        WGNGCGLFGKGSLVTCAKFTC--SKKMTGKSIQPENLEYRIMLSVHGSQH
Zika_virus_2                        WGNGCGLFGKGSLVTCAKFTC--SKKMTGKSIQPENLEYRIMLSVHGSQH
Zika_virus_3                        WGNGCGLFGKGSLVTCAKFTC--SKKMTGKSIQPENLEYRIMLSVHGSQH
Zika_virus_4                        WGNGCGLFGKGSLVTCAKFTC--SKKMTGKSIQPENLEYRIMLSVHGSQH
Dengue_virus_1_1                    WGNGCGLFGKGSLITCAKFKC--VTKLEGKIVQYENLKYSVIVTVHTGDQ
Dengue_virus_1_2                    WGNGCGLFGKGSLLTCAKFKC--VTKLEGKIVQYENLKYSVIVTVHTGDQ
Dengue_virus_1_3                    WGNGCGLFGKGSLITCAKFKC--VTKLEGKIVQYENLKYSVIVTVHTGDQ
Dengue_virus_1_4                    WGNGCGLFGKGSLITCAKFKC--VTKLEGKIVQYENLKYSVIVTVHTGDQ
Dengue_virus_2_1                    WGNGCGLFGKGGIVTCAMFTC--KKNMEGKIVQPENLEYTIVVTPHSGEE
Dengue_virus_2_2                    WGNGCGLFGKGGIVTCAMFTC--KKNMEGKVVQPENLEYTIVITPHSGEE
Dengue_virus_2_3                    WGNGCGLFGKGGIVTCAMFTC--KKNMEGKVVQPENLEYTIVITPHSGEE
Dengue_virus_2_4                    WGNGCGLFGKGGIVTCAMFTC--KKNMEGKVVLPENLEYTIVITPHSGEE
Dengue_virus_3_1                    WGNGCGLFGKGSLVTCAKFQC--LELIEGKVVQHENLKYTVIITVHTGDQ
Dengue_virus_3_2                    WGNGCGLFGKGSLVTCAKFQC--LESIEGKVVQHENLKYTVTITVHTGDQ
Dengue_virus_3_3                    WGNGCGLFGKGSLVTCAKFQC--LEPIEGKVVQYENLKYTVIITVHTGDQ
Dengue_virus_3_4                    WGNGCGLFGKGSLVTCAKFQC--LESIEGKVVQHENLKYTVIITVHTGDQ
Dengue_virus_4_1                    WGNGCGLFGKGGVVTCAKFSC--SGKITGNLVQIENLEYTVVVTVHNGDT
Dengue_virus_4_2                    WGNGCGLFGKGGVVTCAKFSC--SGKITGNLVQIENLEYTVVVTVHNGDT
Dengue_virus_4_3                    WGNGCGLFGKGGVVTCAKFLC--LGKITGNLVQIENLEYTVVVTVHNGDT
Dengue_virus_4_4                    WGNGCGLFGKGGVVTCAKFLC--SGKITGNLVQIENLEYTVVVTVHNGDT
Japanese_encephalitis_virus_1       WGNGCGLFGKGSIDTCAKFSC--TSKAIGRTIQPENIKYEVGIFVHGTTT
Japanese_encephalitis_virus_2       WGNGCGLFGKGSIDTCAKFSC--TSKAIGRTIQPENIKYEVGIFVHGTTT
Japanese_encephalitis_virus_3       WGNGCGLFGKGSIDTCAKFSC--TSKAIGRMIQPENIKYEVGIFVHGTTT
Japanese_encephalitis_virus_4       WGNGCGLFGKGSIDTCAKFSC--TSKAIGRMIQPENIKYEVGIFVHGTTT
West_Nile_virus_1                   WGNGCGLFGKGSIDTCAKFAC--STNATGRTILKENIKYEVAIFVHGPTT
West_Nile_virus_2                   WGNGCGLFGKGSIDTCAKFAC--TTKATGWIIQKENIKYEVAIFVHGPTT
West_Nile_virus_3                   WGNGCGLFGKGSIDTCAKFAC--STKAIGRTILKENIKYEVAIFVHGPTT
West_Nile_virus_4                   WGNGCGLFGKGSIDTCAKFAC--STKAIGRTILKENIKYEVAIFVHGPTT
Yellow_fever_virus_1                WGNGCGLFGKGSIVACAKFTC--AKSMSLFEVDQTKIQYVIRAQLHVGAK
Yellow_fever_virus_2                WGNGCGLFGKGSIVACAKFTC--AKSMSLFEVDQTKIQYVIRAQLHVGAK
Yellow_fever_virus_3                WGNGCGLFGKGSIVACAKFTC--AKSMSLFEVDQTKIQYVIRAQLHVGAK
Yellow_fever_virus_4                WGNGCGLFGKGSIVACAKFAC--AKSMSLFEVDQTKIQYVIRAQLHVGAK
#=GC RF                             xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
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```
Zika_virus_1                        SGMIV------.NDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDF
Zika_virus_2                        SGMIV------.NDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDF
Zika_virus_3                        SGMIV------.NDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDF
Zika_virus_4                        SGMIV------.NDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDF
Dengue_virus_1_1                    HQVGNE-----.STEHGTTATITPQAPTSEIQLTDYGALTLDCSPRTGLDF
Dengue_virus_1_2                    HQVGNE-----.TTEHGTIATITPQAPTSEIQLTDYGALTLDCSPRTGLDF
Dengue_virus_1_3                    HQVGNE-----.STEHGTTATITPQAPTTEIQLTDYGALTLDCSPRTGLDF
Dengue_virus_1_4                    HQVGNE-----.STEHGTTATITPQAPTTEIQLTDYGALTLDCSPRTGLDF
Dengue_virus_2_1                    HAVGND-----.TGKHGKEIKVTPQSSITEAELTGYGTVTMECSPRTGLDF
Dengue_virus_2_2                    HAVGND-----.TGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDF
Dengue_virus_2_3                    HAVGND-----.TGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDF
Dengue_virus_2_4                    HAVGND-----.TGKHGKEIKITPQSSITEAELTGYGTVTMECSPRTGLDF
Dengue_virus_3_1                    HQVG-------.NETQGVTAEITPQASTVEAILPEYGTLGLECSPRTGLDF
Dengue_virus_3_2                    HQVGND-----.--TQGVTAEITPQASTVEAILPEYGTLGLECSPRTGLDF
Dengue_virus_3_3                    HQVG-------.NETQGVTAEITPQASTTEAILPEYGTLGLECSPRTGLDF
Dengue_virus_3_4                    HQVG-------.NETQGVTAEITPQASTVEAILPEYGTLGLECSPRTGLDF
Dengue_virus_4_1                    HAVGND-----.TSNHGVTATITPRSPSVEVKLPDYGELTLDCEPRSGIDF
Dengue_virus_4_2                    HAVGND-----.TSNHGVTATITPRSPSVEVELPDYGELTLDCEPRSGIDF
Dengue_virus_4_3                    HAVGND-----.TSNHGVTATITPRSPSVEVKLPDYGELTLDCEPRSGIDF
Dengue_virus_4_4                    HAVGND-----.TSNHGVTATITPRSPSVEVKLPDYGELTLDCEPRSGIDF
Japanese_encephalitis_virus_1       SENHGNYSAQvGASQAAKFTVTPNAPSITLKLGDYGEVTLDCEPRSGLNT
Japanese_encephalitis_virus_2       SENHGNYSAQvGASQAAKFTVTPNAPSITLKLGDYGEVTLDCEPRSGLNT
Japanese_encephalitis_virus_3       SENHGNYSAQvGASQAAKFTVTPNAPSITLKLGDYGEVTLDCEPRSGLNT
Japanese_encephalitis_virus_4       SENHGNYSAQvGASQAAKFTVTPNAPSITLKLGDYGEVTLDCEPRSGLNT
West_Nile_virus_1                   VESHGNYSTQtGATQAGRFGITPAAPSYTLKLGEYGEVTVDCEPRSGIDT
West_Nile_virus_2                   VESHGNYSTQmGATQAGRFSITPSAPSYTLKLGEYGEVTVDCEPRSGIDT
West_Nile_virus_3                   VESHGNYSTQaGATQAGRFSITPAAPSYTLKLGEYGEVTVDCEPRSGIDT
West_Nile_virus_4                   VESHGNYSTQaGATQAGRFSITPAAPSYTLKLGEYGEVTVDCEPRSGIDT
Yellow_fever_virus_1                QEN--------.WNTDIKTLKFDALSGSQEAEFTGYGRATLECQVQTAVDF
Yellow_fever_virus_2                QEN--------.WNTDIKTLKFDALSGSQEAEFTGYGRATLECQVQTAVDF
Yellow_fever_virus_3                QEN--------.WNTDIKTLKFDALSGSQEAEFTGYGKATLECQVQTAVDF
Yellow_fever_virus_4                QEN--------.WNTDIKTLKFDALSGSQEAEFTGYGRATLECQVQTAVDF
#=GC RF                             xxxxxxxxxx.xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
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Flavivirus glycoprotein, central and dimerisation domains

ANNEX

HMM alignment

```
Zika_virus_1                          SDLYYLTMN------NKHWLVHKEWFHDIPLPWHAGADTGTpHWNNKEALV
Zika_virus_2                          SDLYYLTMN------NKHWLVHKEWFHDIPLPWHAGADTGTpHWNNKEALV
Zika_virus_3                          SDLYYLTMN------NKHWLVHKEWFHDIPLPWHAGADTGTpHWNNKEALV
Zika_virus_4                          SDLYYLTMN------NKHWLVHKEWFHDIPLPWHAGADTGTpHWNNKEALV
Dengue_virus_1_1                      NEMVLLTMK------EKSWLVHKQWFLDLPLPWTSGASTSQeTWNRQDLLV
Dengue_virus_1_2                      NEMVLLTMK------EKSWLVHKQWFLDLPLPWTSGASTSQeTWNRQDLLV
Dengue_virus_1_3                      NEMVLLTMK------EKSWLVHKQWFLDLPLPWTSGASTSQeTWNRQDLLV
Dengue_virus_1_4                      NEMVLLTMK------EKSWLVHKQWFLDLPLPWTSGASTSQeTWNRQDLLV
Dengue_virus_2_1                      NEMVLLQME------NKAWLVHRQWFLDLPLPWLPGADKQEsNWIQKETLV
Dengue_virus_2_2                      NEMVLLQME------DKAWLVHRQWFLDLPLPWLPGADTQGsNWIQKETLV
Dengue_virus_2_3                      NEMVLLQME------DKAWLVHRQWFLDLPLPWLPGADTQGsNWIQKETLV
Dengue_virus_2_4                      NEMVLLQME------DKAWLVHRQWFLDLPLPWLPGADTQGsNWIQKETLV
Dengue_virus_3_1                      NEMILLTMK------NKAWMVHRQWFFDLPLPWTSGATTETpTWNKKELLV
Dengue_virus_3_2                      NEMILLTMK------NKAWMVHRQWFFDLPLPWTSGATTETpTWNRKELLV
Dengue_virus_3_3                      NEMILLTMK------NKAWMVHKQWFFDLPLPWTSGATTETpTWNRKELLV
Dengue_virus_3_4                      NEMILLTMK------NKAWMVHRQWFFDLPLPWTSGATTETpTWNKKELLV
Dengue_virus_4_1                      NEMILMKMK------KKTWLVHKQWFLDLPLPWTTGADTSEvHWNYKERMV
Dengue_virus_4_2                      NEMILMKMN------KKTWLVHKQWFLDLPLPWTAGADTSEvHWNNKERMV
Dengue_virus_4_3                      NEMILMKMK------KKTWLVHKQWFLDLPLPWTAGADTSEvHWNYKERMV
Dengue_virus_4_4                      NEMILMKMK------KKTWLVHKQWFLDLPLPWTAGADTSEvHWNYKERMV
Japanese_encephalitis_virus_1         EAFYVMTVG------SKSFLVHREWFHDLALPWTPPSSTA-.-WRNRELLM
Japanese_encephalitis_virus_2         EAFYVMTVG------SKSFLVHREWFHDLALPWTSPSSTA-.-WRNRELLM
Japanese_encephalitis_virus_3         EAFYVMTVG------SKSFLVHREWFHDLSLPWTSPSSTA-.-WRNRELLM
Japanese_encephalitis_virus_4         EAFYVMTVG------SKSFLVHREWFHDLSLPWTSPSSTA-.-WRNRELLM
West_Nile_virus_1                     NAYYVMTVG------TKTFLVHREWFMDLNLPWSSAGSTV-.-WRNRETLM
West_Nile_virus_2                     SAYYVMSVG------AKSFLVHREWFMDLNLPWSSAGSTT-.-WRNRETLM
West_Nile_virus_3                     NAYYVMTVG------TKTFLVHREWFMDLNLPWSSAGSTV-.-WRNRETLM
West_Nile_virus_4                     NAYYVMTVG------TKTFLVHREWFMDLNLPWSSAGSTV-.-WRNRETLM
Yellow_fever_virus_1                  SNSYIAEME------KESWIVDKQWAQDLTLPWQSGSGGV-.-WREMHHLV
Yellow_fever_virus_2                  SNSYIAEME------KESWIVDKQWAQDLTLPWQSGSGGV-.-WREMHHLV
Yellow_fever_virus_3                  SNSYIAEME------KESWIVDKQWAQDLTLPWQSGSGGV-.-WREMHHLV
Yellow_fever_virus_4                  SNSYIAEME------KESWIVDKQWAQDLTLPWQSGSGGV-.-WREMHHLV
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Zika_virus_1                          EFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAK--.GRLFSGHLK
Zika_virus_2                          EFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAK--.GRLFSGHLK
Zika_virus_3                          EFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAK--.GRLFSGHLK
Zika_virus_4                          EFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAK--.GRLFSGHLK
Dengue_virus_1_1                      TFKTAHAKKQEVVVLGSQEGAMHTALTGATEIQTSGTT--.-TIFAGHLK
Dengue_virus_1_2                      TFKTAHAKKQEVVVLGSQEGAMHTALTGATEIQTSGTT--.-TIFAGHLK
Dengue_virus_1_3                      TFKTAHAKKQEVVVLGSQEGAMHTALTGATEIQTSGTT--.-TIFAGHLK
Dengue_virus_1_4                      TFKTAHAKKQEVVVLGSQEGAMHTALTGATEIQTSGTT--.-TIFAGHLK
Dengue_virus_2_1                      TFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGN--.-LLFTGHLK
Dengue_virus_2_2                      TFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGN--.-LLFTGHLK
Dengue_virus_2_3                      TFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGN--.-LLFTGHLK
Dengue_virus_2_4                      TFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGN--.-LLFTGHLK
Dengue_virus_3_1                      TFKNAHAKKQEVVVLGSQEGAMHTALTGATEIQTSGGT--.-SIFAGHLK
Dengue_virus_3_2                      TFKNAHAKKQEVVVLGSQEGAMHTALTGATEIQNSGGT--.-SIFAGHLK
Dengue_virus_3_3                      TFKNAHAKKQEVVVLGSQEGAMHTALTGATEIQNSGGT--.-SIFAGHLK
Dengue_virus_3_4                      TFKNAHAKKQEVVVLGSQEGAMHTALTGATEIQTSGGT--.-SIFAGHLK
Dengue_virus_4_1                      TFKVPHAKRQDVTVLGSQEGAMHSALAGATEVDSGDGN--.-HMFAGHLK
Dengue_virus_4_2                      TFKVPHAKRQDVTVLGSQEGAMHSALAGATEVDSGDGN--.-HMFAGHLK
Dengue_virus_4_3                      TFKVPHAKRQDVTVLGSQEGAMHSALAGATEVDSGDGN--.-HMFAGHLK
Dengue_virus_4_4                      TFKVPHAKRQDVTVLGSQEGAMHSALAGATEVDSGDGN--.-HMFAGHLK
Japanese_encephalitis_virus_1         EFEEAHATKQSVVALGSQEGGLHQALAGAIVVEYSSSV--.-KLTSGHLK
Japanese_encephalitis_virus_2         EFEEAHATKQSVVALGSQEGSLHQALAGAIVVEYSSSV--.-KLTSGHLK
Japanese_encephalitis_virus_3         EFEEAHATKQSVVALGSQEGGLHQALAGAIVVEYSSSV--.-KLTSGHLK
Japanese_encephalitis_virus_4         EFEEAHATKQSVVALGSQEGGLHQALAGAIVVEYSSSV--.-KLTSGHLK
West_Nile_virus_1                     EFEEPHATKQSVIALGSQEGALHQALAGAIPVEFSSNT--.VKLTSGHLK
West_Nile_virus_2                     EFEEPHATKQSVVALGSQEGALHQALAGAIPVEFSSNT--.VKLTSGHLK
West_Nile_virus_3                     EFEEPHATKQSVIALGSQEGALHQALAGAIPVEFSSNT--.VKLTSGHLK
West_Nile_virus_4                     EFEEPHATKQSVIALGSQEGALHQALAGAIPVEFSSNT--.VKLTSGHLK
Yellow_fever_virus_1                  EFEPPHAATIKVLALGNQEGSLKTALTGAMRVTKDTNNSKlYKLHGGHVA
Yellow_fever_virus_2                  EFEPPHAATIKVLALGNQEGSLKTALTGAMRVTKDTNNSKlYKLHGGHVA
Yellow_fever_virus_3                  EFEPPHAATIKVLALGNQEGSLKTALTGAMRVTKDTNNSKlYKLHGGHVA
Yellow_fever_virus_4                  EFEPPHAATIKVLALGNQEGSLKTALTGAMRVTKDTNNSKlYKLHGGHVA
#=GC RF                               xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx.xxxxxxxxx
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Flavivirus glycoprotein, central and dimerisation domains

ANNEX

HMM alignment

```
Zika_virus_1                          CRLKMDKLRLKGvsyslctaaftftkvpaetlhgtvtvevqyagtdgpck
Zika_virus_2                          CRLKMDKLRLKGvsyslctaaftftkvpaetlhgtvtvevqyagtdgpck
Zika_virus_3                          CRLKMDKLRLKGvsyslctaaftftkvpaetlhgtvtvevqyagtdgpck
Zika_virus_4                          CRLKMDKLRLKGvsyslctaaftftkvpaetlhgtvtvevqyagtdgpck
Dengue_virus_1_1                      CRLKMDKLTLKGmsyvmctgsfklekevaetqhgtvlvqikyegtdapck
Dengue_virus_1_2                      CRLKMDKLTLKGtsyvmctgsfklekevaetqhgtvlvqvkyegtdapck
Dengue_virus_1_3                      CRLKMDKLTLKGmsyvmctgsfklekevaetqhgtvlvqikyegtdapck
Dengue_virus_1_4                      CRLKMDKLTLKGmsyvmctgsfklekevaetqhgtvlvqikyegtdapck
Dengue_virus_2_1                      CRLRMDKLQLKGmsysmctgkfkvvkeiaetqhgtivirvqyegdgspck
Dengue_virus_2_2                      CRLRMDKLQLKGmsysmctgkfkivkeiaetqhgtivirvqyegdgspck
Dengue_virus_2_3                      CRLRMDKLQLKGmsysmctgkfkivkeiaetqhgtivirvqyegdgspck
Dengue_virus_2_4                      CRLRMDKLQLKGmsysmctgkfkivkeiaetqhgtivirvqyegdgspck
Dengue_virus_3_1                      CRLKMDKLELKGmsyamcsnafvlkkevsetqhgtilikveykgedapck
Dengue_virus_3_2                      CRLKMDKLELKGmsyamclntfvlkkevsetqhgtilikveykgedapck
Dengue_virus_3_3                      CRLKMDKLELKGmsyamctntfvlkkevsetqhgtilikveykgedapck
Dengue_virus_3_4                      CRLKMDKLELKGmsyvmcsnafvlkkevsetqhgtilikveykgedapck
Dengue_virus_4_1                      CKVRMEKLRIKGmsytmcsgkfsidkemaetqhgttvvkvkyegagapck
Dengue_virus_4_2                      CKVRMEKLRIKGmsytmcsgkfsidkemaetqhgttvvkvkyegagapck
Dengue_virus_4_3                      CKVRMEKLRIKGmsytmcsgkfsidkemaetqhgttvvkvkyegagapck
Dengue_virus_4_4                      CKVRMEKLRIKGmsytmcsgkfsidkemaetqhgttvvkvkyegagapck
Japanese_encephalitis_virus_1         CRLKMDKLALKGttygmctgkfsfaknpadtghgtvvielsysgsdgpck
Japanese_encephalitis_virus_2         CRLKMDKLALKGttygmctekfsfaknpadtghgtvvielsysgsdgpck
Japanese_encephalitis_virus_3         CRLKMDKLALKGttygmctekfsfaknpadtghgtvvieltysgsdgpck
Japanese_encephalitis_virus_4         CRLKMDKLALKGttygmctekfsfaknpadtghgtvvieltysgsdgpck
West_Nile_virus_1                     CRVKLEKLQLKGttygvcskafkflgtpadtghgtvvlelqytgtdgpck
West_Nile_virus_2                     CRVKMEKLQLKGttygvcskafkfagtpadtghgtvvlelqytgtdgpck
West_Nile_virus_3                     CRVKMEKLQLKGttygvcskafkflgtpadtghgtvvlelqytgtdgpck
West_Nile_virus_4                     CRVKMEKLQLKGttygvcskafkflgtpadtghgtvvlelqytgtdgpck
Yellow_fever_virus_1                  CRVKLSALTLKGtsykmctdkmsfvknptdtghgtavmqvkvpkgapcri
Yellow_fever_virus_2                  CRVKLSALTLKGtsykmctdkmsfvknptdtghgtavmqvkvpkgapcri
Yellow_fever_virus_3                  CRVKLSALTLKGtsykmctdkmsfvknptdtghgtavmqvkvpkgapcri
Yellow_fever_virus_4                  CRVKLSALTLKGtsykmctdkmsfvknptdtghgtavmqvkvpkgapcri
#=GC RF                               xxxxxxxxxxxx.....................................
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Zika_virus_1                          vpaqmavdmqtltpvgrlitanpvitestenskmmleldppfgdsyivig
Zika_virus_2                          vpaqmavdmqtltpvgrlitanpvitestenskmmleldppfgdsyivig
Zika_virus_3                          vpaqmavdmqtltpvgrlitanpvitestenskmmleldppfgdsyivig
Zika_virus_4                          vpaqmavdmqtltpvgrlitanpvitestenskmmleldppfgdsyivig
Dengue_virus_1_1                      ipfstqdekgvtqngrlitanpivtdkekpvnieaeppfgesyivigage
Dengue_virus_1_2                      ipfstqdekgvtqngrlitanpivtdkekpvnieteppfgesyivvgage
Dengue_virus_1_3                      ipfstqdekgvtqngrlitanpivtdkekpvnieaeppfgesyivigage
Dengue_virus_1_4                      ipfstqdekgvtqngrlitanpivtdkekpvnieaeppfgesyivigage
Dengue_virus_2_1                      ipfeimdlekryvlgrlitvnpivtekdspvnieaeppfgdsyiiigvep
Dengue_virus_2_2                      ipfeitdlekrhvlgrlitvnpivtekdspvnieaeppfgdsyiiigvep
Dengue_virus_2_3                      ipfeitdlekrhvlgrlitvnpivtekdspvnieaeppfgdsyiiigvep
Dengue_virus_2_4                      ipfeimdlekrhvlgrlitvnpivtekdspvnieaeppfgdsyiiigvep
Dengue_virus_3_1                      ipfstedeqgkahngrlitanpvvtkkeepvnieaeppfgesniiigigd
Dengue_virus_3_2                      ipfstedgqgkahngrlitanpvvtkkeepvnieaeppfgesnivigigd
Dengue_virus_3_3                      ipfstedgqgkahngrlitanpvvtkkeepvnieaeppfgesnivigigd
Dengue_virus_3_4                      ipfstedgqgkahngrlitanpvvtkkeepvnieaeppfgesniiigigd
Dengue_virus_4_1                      vpieirdvnkekvvgrvisatplaentnsvtnieleppfgdsyivigvgn
Dengue_virus_4_2                      vpieirdvnkekvigrvissiplaentnsvtnieleppfgdsyivigvgn
Dengue_virus_4_3                      ipieirdvnkekvvgriisstpfaentnsvtnieleppfgdsyivigvgd
Dengue_virus_4_4                      ipieirdvnkekvvgriisstpfaentnsvtnieleppfgdsyivigvgd
Japanese_encephalitis_virus_1         ipivsvaslndmtpvgrlvtvnpfvatssanskvlvemeppfg.......
Japanese_encephalitis_virus_2         ipivsvaslndmtpvgrlvtvnpfvatssanskvlvemeppfgdsyivvg
Japanese_encephalitis_virus_3         ipivsvaslndmtpvgrlvtvnpfvatsssnskvlvemeppfgdsyivvg
Japanese_encephalitis_virus_4         ipivsvaslndmtpvgrlvtvnpfvatsssnskvlvemeppfgdsyivvg
West_Nile_virus_1                     vpissvaslndltpvgrlvtvnpfvsvstanakvlieleppfgdsyivvg
West_Nile_virus_2                     vpissvaslndltpvgrlvtvnpfvsvatanskvlieleppfgdsyivvg
West_Nile_virus_3                     vpissvaslndltpvgrlvtvnpfvsvatanakvlieleppfgdsyivvg
West_Nile_virus_4                     vpissvaslndltpvgrlvtvnpfvsvatanakvlieleppfgdsyivvg
Yellow_fever_virus_1                  pvmvaddltaavnkgilvtvnpiastnddevlievnppfgdsyiivgtgd
Yellow_fever_virus_2                  pvmvaddltaavnkgilvtvnpiastnddevlievnppfgdsyiivgtgd
Yellow_fever_virus_3                  pvmvaddltaavnkgilvtvnpiastnddevlievnppfgdsyiivgtgd
Yellow_fever_virus_4                  pvmvaddltaavnkgilvtvnpiastnddevlievnppfgdsyiivgtgd
#=GC RF                               ................................................
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Flavivirus glycoprotein, central and dimerisation domains

ANNEX

HMM alignment

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Zika_virus_1                      vgdkkithhwhrsgstigkafeatvrgakrmavlgdtawdfgsvggvfns
Zika_virus_2                      vgdkkithhwhrsgstigkafeatvrgakrmavlgdtawdfgsvggvfns
Zika_virus_3                      vgdkkithhwhrsgstigkafeatvrgakrmavlgdtawdfgsvggvfns
Zika_virus_4                      vgdkkithhwhrsgstigkafeatvrgakrmavlgdtawdfgsvggvfns
Dengue_virus_1_1                  kalklswfkkgssigkmfeatargarrmailgdtawdfgsiggvftsvgk
Dengue_virus_1_2                  kalklswfkkgssigkmfeatargarrmailgdtawdfgsiggvftsvgk
Dengue_virus_1_3                  kalklswfkkgssigkmfeatargarrmailgdtawdfgsiggvftsvgk
Dengue_virus_1_4                  kalklswfkkgssigkmfeatargarrmailgdtawdfgsiggvftsvgk
Dengue_virus_2_1                  gqlklnwfkkgssigqmfettmrgakrmailgdtawdfgslggvftsvgk
Dengue_virus_2_2                  gqlklnwfkkgssigqmfettmrgakrmailgdtawdfgslggvftsigk
Dengue_virus_2_3                  gqlklnwfkkgssigqmfettmrgakrmailgdtawdfgslggvftsigk
Dengue_virus_2_4                  gqlklnwfkkgssigqmfettmrgakrmailgdtawdfgslggvftsigk
Dengue_virus_3_1                  kalkinwykkgssigkmfeatargarrmailgdtawdfgsvggvlnslgk
Dengue_virus_3_2                  kalkinwykkgssigkmfeatargarrmailgdtawdfgsvggvlnslgk
Dengue_virus_3_3                  nalkinwykkgssigkmfeatargarrmailgdtawdfgsvggvlnslgk
Dengue_virus_3_4                  kalkinwykkgssigkmfeatargarrmailgdtawdfgsvggvlnslgk
Dengue_virus_4_1                  saltlhwfrkgssigkmfestyrgakrmailgetawdfgsvgglftslgk
Dengue_virus_4_2                  saltlhwfrkgssigkmfestyrgakrmailgetawdfgsvgglftslgk
Dengue_virus_4_3                  saltlhwfrkgssigkmfestyrgakrmailgetawdfgsvgglftslgk
Dengue_virus_4_4                  saltlhwfrkgssigkmfestyrgakrmailgetawdfgsvgglftslgk
Japanese_encephalitis_virus_1     ................................................
Japanese_encephalitis_virus_2     rgdkqinhhwhkagstlgkafsttlkgaqrlaalgdtawdfgsiggvfns
Japanese_encephalitis_virus_3     rgdkqinhhwhkagstlgkafsttlkgaqrlaalgdtawdfgsiggvfns
Japanese_encephalitis_virus_4     rgdkqinhhwhkagstlgkafsttlkgaqrlaalgdtawdfgsiggvfns
West_Nile_virus_1                 rgeqqinhhwhksgssigkaftttlkgaqrlaalgdtawdfgsvggvfts
West_Nile_virus_2                 rgeqqinhhwhksgssigkaftttlrgaqrlaalgdtawdfgsvggvfas
West_Nile_virus_3                 rgeqqinhhwhksgssigkaftttlkgaqrlaalgdtawdfgsvggvfts
West_Nile_virus_4                 rgeqqinhhwhksgssigkaftttlkgaqrlaalgdtawdfgsvggvfts
Yellow_fever_virus_1              srltyqwhkegssigklftqtmkgaerlavmgdaawdfgsaggfftsvgk
Yellow_fever_virus_2              srltyqwhkegssigklftqtmkgaerlavmgdaawdfgsaggfftsvgk
Yellow_fever_virus_3              srltyqwhkegssigklftqtmkgaerlavmgdaawdfgsaggfftsvgk
Yellow_fever_virus_4              srltyqwhkegssigklftqtmkgaerlavmgdaawdfgsaggfftsvgk
#=GC RF                           ................................................
```

```
Zika_virus_1                      lgkgihqifgaafkslfggmswfsqiligtllvwlglntkngsisltcla
Zika_virus_2                      lgkgihqifgaafkslfggmswfsqiligtllvwlglntkngsisltcla
Zika_virus_3                      lgkgihqifgaafkslfggmswfsqiligtllvwlglntkngsisltcla
Zika_virus_4                      lgkgihqifgaafkslfggmswfsqiligtllvwlglntkngsisltcla
Dengue_virus_1_1                  lvhqifgtaygvlfsgvswtmkigigvlltwlglnsrstslsmtciavgl
Dengue_virus_1_2                  lvhqvfgtaygvlfsgvswtmkigigilltwlglnsrstslsmtciavgm
Dengue_virus_1_3                  lvhqifgtaygvlfsgvswtmkigigvlltwlglnsrstsfsmtciavgl
Dengue_virus_1_4                  lvhqifgtaygvlfsgvswtmkigigvlltwlglnsrstslsmtciavgl
Dengue_virus_2_1                  alhqvfgaiygaafsgvswtmkiligviitwigmnsrstslsvslvlvgi
Dengue_virus_2_2                  alhqvfgaiygaafsgvswtmkiligviitwigmnsrstslsvslvlvgv
Dengue_virus_2_3                  alhqvfgaiygaafsgvswtmkiligviitwigmnsrstslsvslvlvgv
Dengue_virus_2_4                  alhqvfgaiygaafsgvswtmkiligviitwigmnsrstslsvslvlvgv
Dengue_virus_3_1                  mvhqifgsaytalfsgvswimkigigvlltwiglnskntsmsfscivigi
Dengue_virus_3_2                  mvhqifgsaytalfggvswimkigigvlltwiglnskntsmsfscivigi
Dengue_virus_3_3                  mvhqifgsaytalfsgvswvmkigigvlltwiglnskntsmsfsciaigi
Dengue_virus_3_4                  mvhqifgsaytalfsgvswimkigigvlltwiglnskntsmsfscivigi
Dengue_virus_4_1                  avhqvfgsvyttmfggvswmiriligflvlwigtnsrntsmamtciavgg
Dengue_virus_4_2                  avhqvfgsvyttmfggvswmiriligflvlwigtnsrntsmamtciavgg
Dengue_virus_4_3                  avhqvfgsvyttmfggvswmvriligflvlwigtnsrntsmamtciavgg
Dengue_virus_4_4                  avhqvfgsvyttmfggvswmvriligflvlwigtnsrntsmamtciavgg
Japanese_encephalitis_virus_1     ................................................
Japanese_encephalitis_virus_2     igkavhqvfggafrtlfggmswitqglmgalllwmgvnardrsialafla
Japanese_encephalitis_virus_3     igkavhqvfgg.....................................
Japanese_encephalitis_virus_4     igkavhqvfgg.....................................
West_Nile_virus_1                 vgkavhqvfggafrslfggmswitqgllgalllwmginardrsialtfla
West_Nile_virus_2                 vgkaihqvfggafrslfggmswitqgllgalllwmginardrsiamtfla
West_Nile_virus_3                 vgkavhqvfggafrslfggmswitqgllgalllwmginardrsialtfla
West_Nile_virus_4                 vgkavhqvfggafrslfggmswitqgllgalllwmginardrsialtfla
Yellow_fever_virus_1              gihtvfgsafqglfgglswitkvimgvvliwvgintrnmtmsmsmilvgv
Yellow_fever_virus_2              gihtvfgsafqglfgglswitkvimgvvliwvgintrnmtmsmsmilvgv
Yellow_fever_virus_3              gihtvfgsafqglfgglswitkvimgvvliwvgintrnmtmsmsmilvgv
Yellow_fever_virus_4              gihtvfgsafqglfgglswitkvimgvvliwvgintrnmtmsmsmilvgv
#=GC RF                           ................................................
```

Flavivirus glycoprotein, central and dimerisation domains

ANNEX

HMM alignment

```
Zika_virus_1                        lggvmiflstavsa
Zika_virus_2                        lggvmiflstavsa
Zika_virus_3                        lggvmiflstavsa
Zika_virus_4                        lggvmiflstavsa
Dengue_virus_1_1                    vtlylgvmvqa...
Dengue_virus_1_2                    vtlylgvmvqa...
Dengue_virus_1_3                    vtlylgvmvqa...
Dengue_virus_1_4                    vtlylgvmvqa...
Dengue_virus_2_1                    vtlylgvmvqa...
Dengue_virus_2_2                    vtlylgamvqa...
Dengue_virus_2_3                    vtlylgavvqa...
Dengue_virus_2_4                    vtlylgvmvqa...
Dengue_virus_3_1                    itlylgavvqa...
Dengue_virus_3_2                    itlylgtvvqa...
Dengue_virus_3_3                    itlylgavvqa...
Dengue_virus_3_4                    itlylgavvqa...
Dengue_virus_4_1                    itlflgftvqa...
Dengue_virus_4_2                    itlflgftvqa...
Dengue_virus_4_3                    itlflgftvqa...
Dengue_virus_4_4                    itlflgftvqa...
Japanese_encephalitis_virus_1       ..............
Japanese_encephalitis_virus_2       tggvlvflatnvha
Japanese_encephalitis_virus_3       ..............
Japanese_encephalitis_virus_4       ..............
West_Nile_virus_1                   vggvllflsvnvha
West_Nile_virus_2                   vggvllflsvnvha
West_Nile_virus_3                   vggvllflsvnvha
West_Nile_virus_4                   vggvllflsvnvha
Yellow_fever_virus_1                immflslgvga...
Yellow_fever_virus_2                immflslgvga...
Yellow_fever_virus_3                immflslgvga...
Yellow_fever_virus_4                immflslgvga...
#=GC RF                             ..............
```

Flavivirus glycoprotein, immunoglobulin-like domain

ANNEX

HMM alignment

```
Zika_virus_1                          ...ircigvsnrdfvegmsggtwvdvvlehggcvtvmaqdkptvdielvt
Zika_virus_2                          ...ircigvsnrdfvegmsggtwvdvvlehggcvtvmaqdkptvdielvt
Zika_virus_3                          ...ircigvsnrdfvegmsggtwvdvvlehggcvtvmaqdkptvdielvt
Zika_virus_4                          ...ircigvsnrdfvegmsggtwvdvvlehggcvtvmaqdkptvdielvt
Dengue_virus_1_1                      ...mrcvgigsrdfveglsgatwvdvvlehgscvttmakdkptldiellk
Dengue_virus_1_2                      ...mrcvgignrdfveglsgatwvdvvlehgscvttmaknkptldiellk
Dengue_virus_1_3                      ...mrcvgigsrdfveglsgatwvdvvlehgscvttmakdkptldiellk
Dengue_virus_1_4                      ...mrcvgigsrdfveglsgatwvdvvlehgscvttmaknkptldiellk
Dengue_virus_2_1                      ...mrcigisnrdfvegvsggswvdivlehgscvttmaknkptldfelik
Dengue_virus_2_2                      ...mrcigisnrdfvegvsggswvdivlehgscvttmaknkptldfelik
Dengue_virus_2_3                      ...mrcigisnrdfvegvsggswvdivlehgscvttmaknkptldfelik
Dengue_virus_2_4                      ...mrcigisnrdfvegvsggswvdivlehgscvttmaknkptldfelik
Dengue_virus_3_1                      .....mrcvgvgnrdfveglsgatwvdvvlehggcvttmaknkptldiel
Dengue_virus_3_2                      .....mrcvgvgnrdfveglsgatwvdvvlehggcvttmaknkptldiel
Dengue_virus_3_3                      ......rcvgvgnrdfveglsgatwvdvvlehggcvttmaknkptldiel
Dengue_virus_3_4                      .....mrcvgvgnrdfveglsgatwvdvvlehggcvttmaknkptldiel
Dengue_virus_4_1                      ...mrcvgvgnrdfvegvsggawvdlvlehggcvttmaqgkptldfeltk
Dengue_virus_4_2                      ...mrcvgvgnrdfvegvsggawvdlvlehggcvttmaqgrptldfeltk
Dengue_virus_4_3                      ...mrcvgvgnrdfvegvsggawvdlvlehggcvttmaqgkptldfeltk
Dengue_virus_4_4                      ...mrcvgvgnrdfvegvsggawvdlvlehggcvttmaqgkptldfeltk
Japanese_encephalitis_virus_1         .fnclgmgnrdfiegasgatwvdlvlegdscltimandkptldvrminie
Japanese_encephalitis_virus_2         ..nclgmgnrdfiegasgatwvdlvlegdscltimandkptldvrminie
Japanese_encephalitis_virus_3         ............egasgatwvdlvlegdscltimandkptldvrminie
Japanese_encephalitis_virus_4         ............egasgatwvdlvlegdscltilandkptldvrminie
West_Nile_virus_1                     fnclgmsnrdflegvsgatwvdlvlegdscvtimskdkptidvkmmnmea
West_Nile_virus_2                     fnclgmsnrdflegvsgatwvdlvlegdscvtimskdkptidvkmmnmea
West_Nile_virus_3                     fnclgmsnrdflegvsgatwvdlvlegdscvtimskdkptidvkmmnmea
West_Nile_virus_4                     fnclgmsnrdflegvsgatwvdlvlegdscvtimskdkptidvkmmnmea
Yellow_fever_virus_1                  .....ahcigitdrdfiegvhggtwvsatleqdkcvtvmapdkpsldisl
Yellow_fever_virus_2                  .....ahcigitdrdfiegvhggtwvsatleqdkcvtvmapdkpsldisl
Yellow_fever_virus_3                  .....ahcigitdrdfiegvhggtwvsatleqdkcvtvmapdkpsldisl
Yellow_fever_virus_4                  .....ahcigitdrdfiegvhggtwvsatleqdkcvtvmapdkpsldisl
#=GC RF                               ................................................
```

```
Zika_virus_1                          ttvsnmaevrsycyeasisdmasdsrcptqgeayldkqsdtqyvckrtlv
Zika_virus_2                          ttvsnmaevrsycyeasisdmasdsrcptqgeayldkqsdtqyvckrtlv
Zika_virus_3                          ttvsnmaevrsycyeasisdmasdsrcptqgeayldkqsdtqyvckrtlv
Zika_virus_4                          ttvsnmaevrsycyeasisdmasdsrcptqgeayldkqsdtqyvckrtlv
Dengue_virus_1_1                      tevtnpavlrklcieakisntttdsrcptqgeatlveeqdanfvcrrtfv
Dengue_virus_1_2                      tevtnpavlrklcieakisntttdsrcptqgeatlveeqdanfvcrrtfv
Dengue_virus_1_3                      tevtnpailrklcieakisntttdsrcptqgeatlveeqdanfvcrrtfv
Dengue_virus_1_4                      tevtnpavlrklcieakisntttdsrcptqgeatlveeqdanfvcrrtfv
Dengue_virus_2_1                      teakqpatlrkycieakltnttesrcptqgepslkeeqdkrfvckhsmv
Dengue_virus_2_2                      teakqpatlrkycieakltnttesrcptqgepslneeqdkrfickhsmv
Dengue_virus_2_3                      teakqpatlrkycieakltnttesrcptqgepslneeqdkrfickhsmv
Dengue_virus_2_4                      teakqpatlrkycieakltnttesrcptqgepslneeqdkrfickhsmv
Dengue_virus_3_1                      qkteatqlatlrklciegkitnvttdsrcptqgeailpeeqdqnyvckht
Dengue_virus_3_2                      qkteatqlatlrklciegkitnittdsrcptqgeailpeeqdqnyvckht
Dengue_virus_3_3                      qkteatqlatlrklciegkitnittdsrcptqgeavlpeeqdqnyvckht
Dengue_virus_3_4                      qkteatqlatlrklciegkitnvttdsrcptqgeailpeeqdqnyvckht
Dengue_virus_4_1                      ttakevallrtyciealisnittatrcptqgepylkeeqdqqyicrrdvv
Dengue_virus_4_2                      ttakevallrtycieasisnittatrcptqgepylkeeqdqqyicrrdvv
Dengue_virus_4_3                      ttakevallrtycieasisnittatrcptqgepylkeeqdqqyicrrdvv
Dengue_virus_4_4                      ttakevallrtycieasisnittatrcptqgepylkeeqdqqyicrrgvv
Japanese_encephalitis_virus_1         asqlaevrsycyhasvtdistvarcpmtgeahnekradssyvckqgftdr
Japanese_encephalitis_virus_2         asqlaevrsycyhasvtdistvarcpttgeahnekradssyvckqgftdr
Japanese_encephalitis_virus_3         asqlaevrsycyhasvtdistvarcpttgeahnekradssyvckqgftdr
Japanese_encephalitis_virus_4         asqlaevrsycyhasvtdistvarcpttgeahnekradssyvckqgftdr
West_Nile_virus_1                     anlaevrsycylaavsdlstkaacptmgeahndkradpafvcrqgvvdrg
West_Nile_virus_2                     anladvrsycylasvsdlstraacptmgeahnekradpafvckqgvvdrg
West_Nile_virus_3                     anlaevrsycylatvsdlstkaacptmgeahndkradpafvcrqgvvdrg
West_Nile_virus_4                     anlaevrsycylatvsdlstkaacptmgeahndkradpafvcrqgvvdrg
Yellow_fever_virus_1                  etvaidgpaearkvcysavltnvkindkcpstgeahleeenegdnackrt
Yellow_fever_virus_2                  etvaidgpaearkvcysavltnvkindkcpstgeahleeenegdnackrt
Yellow_fever_virus_3                  etvaidgpaearkvcysavltnvkindkcpstgeahleeenegdnackrt
Yellow_fever_virus_4                  etvaidgpaearkvcysavltnvkindkcpstgeahleeenegdnackrt
#=GC RF                               ................................................
```

Flavivirus glycoprotein, immunoglobulin-like domain

ANNEX

HMM alignment

```
Zika_virus_1                          drgwgngcglfgkgslvtcakftcskkmtgksiqpenleyrimlsvhgsq
Zika_virus_2                          drgwgngcglfgkgslvtcakftcskkmtgksiqpenleyrimlsvhgsq
Zika_virus_3                          drgwgngcglfgkgslvtcakftcskkmtgksiqpenleyrimlsvhgsq
Zika_virus_4                          drgwgngcglfgkgslvtcakftcskkmtgksiqpenleyrimlsvhgsq
Dengue_virus_1_1                      drgwgngcglfgkgslitcakfkcvtklegkivqyenlkysvivtvhtgd
Dengue_virus_1_2                      drgwgngcglfgkgslltcakfkcvtklegkivqyenlkysvivtvhtgd
Dengue_virus_1_3                      drgwgngcglfgkgslitcakfkcvtklegkivqyenlkysvivtvhtgd
Dengue_virus_1_4                      drgwgngcglfgkgslitcakfkcvtklegkivqyenlkysvivtvhtgd
Dengue_virus_2_1                      drgwgngcglfgkggivtcamftckknmegkivqpenleytivvtphsge
Dengue_virus_2_2                      drgwgngcglfgkggivtcamftckknmegkvvqpenleytivitphsge
Dengue_virus_2_3                      drgwgngcglfgkggivtcamftckknmegkvvqpenleytivitphsge
Dengue_virus_2_4                      drgwgngcglfgkggivtcamftckknmegkvvlpenleytivitphsge
Dengue_virus_3_1                      yvdrgwgngcglfgkgslvtcakfqcleliegkvvqhenlkytviitvht
Dengue_virus_3_2                      yvdrgwgngcglfgkgslvtcakfqclesiegkvvqhenlkytvtitvht
Dengue_virus_3_3                      yvdrgwgngcglfgkgslvtcakfqclepiegkvvqyenlkytviitvht
Dengue_virus_3_4                      yvdrgwgngcglfgkgslvtcakfqclesiegkvvqhenlkytviitvht
Dengue_virus_4_1                      drgwgngcglfgkggvvtcakfscsgkitgnlvqienleytvvvtvhngd
Dengue_virus_4_2                      drgwgngcglfgkggvvtcakfscsgkitgnlvqienleytvvvtvhngd
Dengue_virus_4_3                      drgwgngcglfgkggvvtcakflclgkitgnlvqienleytvvvtvhngd
Dengue_virus_4_4                      drgwgngcglfgkggvvtcakflcsgkitgnlvqienleytvvvtvhngd
Japanese_encephalitis_virus_1         gwgngcglfgkgsidtcakfsctskaigrtiqpenikyevgifvhgttts
Japanese_encephalitis_virus_2         gwgngcglfgkgsidtcakfsctskaigrtiqpenikyevgifvhgttts
Japanese_encephalitis_virus_3         gwgngcglfgkgsidtcakfsctskaigrmiqpenikyevgifvhgttts
Japanese_encephalitis_virus_4         gwgngcglfgkgsidtcakfsctskaigrmiqpenikyevgifvhgttts
West_Nile_virus_1                     wgngcglfgkgsidtcakfacstnatgrtilkenikyevaifvhgpttve
West_Nile_virus_2                     wgngcglfgkgsidtcakfacttkatgwiiqkenikyevaifvhgpttve
West_Nile_virus_3                     wgngcglfgkgsidtcakfacstkaigrtilkenikyevaifvhgpttve
West_Nile_virus_4                     wgngcglfgkgsidtcakfacstkaigrtilkenikyevaifvhgpttve
Yellow_fever_virus_1                  ysdrgwgngcglfgkgsivacakftcaksmslfevdqtkiqyviraqlhv
Yellow_fever_virus_2                  ysdrgwgngcglfgkgsivacakftcaksmslfevdqtkiqyviraqlhv
Yellow_fever_virus_3                  ysdrgwgngcglfgkgsivacakftcaksmslfevdqtkiqyviraqlhv
Yellow_fever_virus_4                  ysdrgwgngcglfgkgsivacakfacaksmslfevdqtkiqyviraqlhv
#=GC RF                               ..................................................
```

```
Zika_virus_1                          ltmnnkhwlvhkewfhdiplpwhagadtgtphwnnkealvefkdahakrq
Zika_virus_2                          ltmnnkhwlvhkewfhdiplpwhagadtgtphwnnkealvefkdahakrq
Zika_virus_3                          ltmnnkhwlvhkewfhdiplpwhagadtgtphwnnkealvefkdahakrq
Zika_virus_4                          ltmnnkhwlvhkewfhdiplpwhagadtgtphwnnkealvefkdahakrq
Dengue_virus_1_1                      lltmkekswlvhkqwfldlplpwtsgastsqetwnrqdllvtfktahakk
Dengue_virus_1_2                      lltmkekswlvhkqwfldlplpwtsgastsqetwnrqdllvtfktahakk
Dengue_virus_1_3                      lltmkekswlvhkqwfldlplpwtsgastsqetwnrqdllvtfktahakk
Dengue_virus_1_4                      lltmkekswlvhkqwfldlplpwtsgastsqetwnrqdllvtfktahakk
Dengue_virus_2_1                      llqmenkawlvhrqwfldlplpwlpgadkqesnwiqketlvtfknphakk
Dengue_virus_2_2                      llqmedkawlvhrqwfldlplpwlpgadtqgsnwiqketlvtfknphakk
Dengue_virus_2_3                      llqmedkawlvhrqwfldlplpwlpgadtqgsnwiqketlvtfknphakk
Dengue_virus_2_4                      llqmedkawlvhrqwfldlplpwlpgadtqgsnwiqketlvtfknphakk
Dengue_virus_3_1                      lltmknkawmvhrqwffdlplpwtsgattetptwnkkellvtfknahakk
Dengue_virus_3_2                      lltmknkawmvhrqwffdlplpwtsgattetptwnrkellvtfknahakk
Dengue_virus_3_3                      lltmknkawmvhkqwffdlplpwtsgattetptwnrkellvtfknahakk
Dengue_virus_3_4                      lltmknkawmvhrqwffdlplpwtsgattetptwnkkellvtfknahakk
Dengue_virus_4_1                      lmkmkkktwlvhkqwfldlplpwttgadtsevhwnykermvtfkvphakr
Dengue_virus_4_2                      lmkmnkktwlvhkqwfldlplpwtagadtsevhwnnkermvtfkvphakr
Dengue_virus_4_3                      lmkmkkktwlvhkqwfldlplpwtagadtsevhwnykermvtfkvphakr
Dengue_virus_4_4                      lmkmkkktwlvhkqwfldlplpwtagadtsevhwnykermvtfkvphakr
Japanese_encephalitis_virus_1         afyvmtvgsksflvhrewfhdlalpwtppsstawrnrellmefeeahatk
Japanese_encephalitis_virus_2         afyvmtvgsksflvhrewfhdlalpwtspsstawrnrellmefeeahatk
Japanese_encephalitis_virus_3         afyvmtvgsksflvhrewfhdlslpwtspsstawrnrellmefeeahatk
Japanese_encephalitis_virus_4         afyvmtvgsksflvhrewfhdlslpwtspsstawrnrellmefeeahatk
West_Nile_virus_1                     yyvmtvgtktflvhrewfmdlnlpwssagstvwrnretlmefeephatkq
West_Nile_virus_2                     yyvmsvgaksflvhrewfmdlnlpwssagsttwrnretlmefeephatkq
West_Nile_virus_3                     yyvmtvgtktflvhrewfmdlnlpwssagstvwrnretlmefeephatkq
West_Nile_virus_4                     yyvmtvgtktflvhrewfmdlnlpwssagstvwrnretlmefeephatkq
Yellow_fever_virus_1                  aemekeswivdkqwaqdltlpwqsgsggvwremhhlvefepphaatikvl
Yellow_fever_virus_2                  aemekeswivdkqwaqdltlpwqsgsggvwremhhlvefepphaatikvl
Yellow_fever_virus_3                  aemekeswivdkqwaqdltlpwqsgsggvwremhhlvefepphaatikvl
Yellow_fever_virus_4                  aemekeswivdkqwaqdltlpwqsgsggvwremhhlvefepphaatikvl
#=GC RF                               ..................................................
```

Flavivirus glycoprotein, immunoglobulin-like domain

ANNEX

HMM alignment

```
Zika_virus_1                      tvvvlgsqegavhtalagaleaemdgakgrlfsghlkcrlkmdklrlkgv
Zika_virus_2                      tvvvlgsqegavhtalagaleaemdgakgrlfsghlkcrlkmdklrlkgv
Zika_virus_3                      tvvvlgsqegavhtalagaleaemdgakgrlfsghlkcrlkmdklrlkgv
Zika_virus_4                      tvvvlgsqegavhtalagaleaemdgakgrlfsghlkcrlkmdklrlkgv
Dengue_virus_1_1                  qevvvlgsqegamhtaltgateiqtsgtttifaghlkcrlkmdkltlkgm
Dengue_virus_1_2                  qevvvlgsqegamhtaltgateiqtsgtttifaghlkcrlkmdkltlkgt
Dengue_virus_1_3                  qevvvlgsqegamhtaltgateiqtsgtttifaghlkcrlkmdkltlkgm
Dengue_virus_1_4                  qevvvlgsqegamhtaltgateiqtsgtttifaghlkcrlkmdkltlkgm
Dengue_virus_2_1                  qdvvvlgsqegamhtaltgateiqmssgnllftghlkcrlkmdklqlkgm
Dengue_virus_2_2                  qdvvvlgsqegamhtaltgateiqmssgnllftghlkcrlrmdklqlkgm
Dengue_virus_2_3                  qdvvvlgsqegamhtaltgateiqmssgnllftghlkcrlrmdklqlkgm
Dengue_virus_2_4                  qdvvvlgsqegamhtaltgateiqmssgnllftghlkcrlrmdklqlkgm
Dengue_virus_3_1                  qevvvlgsqegamhtaltgateiqtsggtsifaghlkcrlkmdklelkgm
Dengue_virus_3_2                  qevvvlgsqegamhtaltgateiqnsggtsifaghlkcrlkmdklelkgm
Dengue_virus_3_3                  qevvvlgsqegamhtaltgateiqnsggtsifaghlkcrlkmdklelkgm
Dengue_virus_3_4                  qevvvlgsqegamhtaltgateiqtsggtsifaghlkcrlkmdklelkgm
Dengue_virus_4_1                  qdvtvlgsqegamhsalagatevdsgdgnhmfaghlkckvrmeklrikgm
Dengue_virus_4_2                  qdvtvlgsqegamhsalagatevdsgdgnhmfaghlkckvrmeklrikgm
Dengue_virus_4_3                  qdvtvlgsqegamhsalagatevdsgdgnhmfaghlkckvrmeklrikgm
Dengue_virus_4_4                  qdvtvlgsqegamhsalagatevdsgdgnhmfaghlkckvrmeklrikgm
Japanese_encephalitis_virus_1     qsvvalgsqegglhqalagaivveysssvkltsghlkcrlkmdklalkgt
Japanese_encephalitis_virus_2     qsvvalgsqegslhqalagaivveysssvkltsghlkcrlkmdklalkgt
Japanese_encephalitis_virus_3     qsvvalgsqegglhqalagaivveysssvkltsghlkcrlkmdklalkgt
Japanese_encephalitis_virus_4     qsvvalgsqegglhqalagaivveysssvkltsghlkcrlkmdklalkgt
West_Nile_virus_1                 svialgsqegalhqalagaipvefssntvkltsghlkcrvkleklqlkgt
West_Nile_virus_2                 svvalgsqegalhqalagaipvefssntvkltsghlkcrvkmeklqlkgt
West_Nile_virus_3                 svialgsqegalhqalagaipvefssntvkltsghlkcrvkmeklqlkgt
West_Nile_virus_4                 svialgsqegalhqalagaipvefssntvkltsghlkcrvkmeklqlkgt
Yellow_fever_virus_1              algnqegslktaltgamrvtkdtnnsklyklhgghvacrvklsaltlkgt
Yellow_fever_virus_2              algnqegslktaltgamrvtkdtnnsklyklhgghvacrvklsaltlkgt
Yellow_fever_virus_3              algnqegslktaltgamrvtkdtnnsklyklhgghvacrvklsaltlkgt
Yellow_fever_virus_4              algnqegslktaltgamrvtkdtnnsklyklhgghvacrvklsaltlkgt
#=GC RF                           ..................................................
```

```
Zika_virus_1                      SYSLCTA-AFTFTKVPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTL
Zika_virus_2                      SYSLCTA-AFTFTKVPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTL
Zika_virus_3                      SYSLCTA-AFTFTKVPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTL
Zika_virus_4                      SYSLCTA-AFTFTKVPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTL
Dengue_virus_1_1                  SYVMCTG-SFKLEKEVAETQHGTVLVQIKYEGTDAPCKIPFSTQD-EKGV
Dengue_virus_1_2                  SYVMCTG-SFKLEKEVAETQHGTVLVQVKYEGTDAPCKIPFSTQD-EKGV
Dengue_virus_1_3                  SYVMCTG-SFKLEKEVAETQHGTVLVQIKYEGTDAPCKIPFSTQD-EKGV
Dengue_virus_1_4                  SYVMCTG-SFKLEKEVAETQHGTVLVQIKYEGTDAPCKIPFSTQD-EKGV
Dengue_virus_2_1                  SYSMCTG-KFKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMD-LEKR
Dengue_virus_2_2                  SYSMCTG-KFKIVKEIAETQHGTIVIRVQYEGDGSPCKIPFEITD-LEKR
Dengue_virus_2_3                  SYSMCTG-KFKIVKEIAETQHGTIVIRVQYEGDGSPCKIPFEITD-LEKR
Dengue_virus_2_4                  SYSMCTG-KFKIVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMD-LEKR
Dengue_virus_3_1                  SYAMCSN-AFVLKKEVSETQHGTILIKVEYKGEDAPCKIPFSTED-EQGK
Dengue_virus_3_2                  SYAMCLN-TFVLKKEVSETQHGTILIKVEYKGEDAPCKIPFSTED-GQGK
Dengue_virus_3_3                  SYAMCTN-TFVLKKEVSETQHGTILIKVEYKGEDAPCKIPFSTED-GQGK
Dengue_virus_3_4                  SYVMCSN-AFVLKKEVSETQHGTILIKVEYKGEDAPCKIPFSTED-GQGK
Dengue_virus_4_1                  SYTMCSG-KFSIDKEMAETQHGTTVVKVKYEGAGAPCKVPIEIRD-VNKE
Dengue_virus_4_2                  SYTMCSG-KFSIDKEMAETQHGTTVVKVKYEGAGAPCKVPIEIRD-VNKE
Dengue_virus_4_3                  SYTMCSG-KFSIDKEMAETQHGTTVVKVKYEGAGAPCKIPIEIRD-VNKE
Dengue_virus_4_4                  SYTMCSG-KFSIDKEMAETQHGTTVVKVKYEGAGAPCKIPIEIRD-VNKE
Japanese_encephalitis_virus_1     TYGMCTG-KFSFAKNPADTGHGTVVIELSYSGSDGPCKIPIVSVASLNDM
Japanese_encephalitis_virus_2     TYGMCTE-KFSFAKNPADTGHGTVVIELSYSGSDGPCKIPIVSVASLNDM
Japanese_encephalitis_virus_3     TYGMCTE-KFSFAKNPADTGHGTVVIELTYSGSDGPCKIPIVSVASLNDM
Japanese_encephalitis_virus_4     TYGMCTE-KFSFAKNPADTGHGTVVIELTYSGSDGPCKIPIVSVASLNDM
West_Nile_virus_1                 TYGVCSK-AFKFLGTPADTGHGTVVLELQYTGTDGPCKVPISSVASLNDL
West_Nile_virus_2                 TYGVCSK-AFKFAGTPADTGHGTVVLELQYTGTDGPCKVPISSVASLNDL
West_Nile_virus_3                 TYGVCSK-AFKFLGTPADTGHGTVVLELQYTGTDGPCKVPISSVASLNDL
West_Nile_virus_4                 TYGVCSK-AFKFLGTPADTGHGTVVLELQYTGTDGPCKVPISSVASLNDL
Yellow_fever_virus_1              SYKMCTD-KMSFVKNPTDTGHGTAVMQVKVP-KGAPCRIPVMVADDLTAA
Yellow_fever_virus_2              SYKMCTD-KMSFVKNPTDTGHGTAVMQVKVP-KGAPCRIPVMVADDLTAA
Yellow_fever_virus_3              SYKMCTD-KMSFVKNPTDTGHGTAVMQVKVP-KGAPCRIPVMVADDLTAA
Yellow_fever_virus_4              SYKMCTD-KMSFVKNPTDTGHGTAVMQVKVP-KGAPCRIPVMVADDLTAA
#=GC RF                           xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
```

Flavivirus glycoprotein, immunoglobulin-like domain

ANNEX

HMM alignment

```
Zika_virus_1                      TPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHR
Zika_virus_2                      TPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHR
Zika_virus_3                      TPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHR
Zika_virus_4                      TPVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHR
Dengue_virus_1_1                  TQNGRLITANPIVTDK--EKPVNIEAEPPFGESYIVIGAGEKALKLSWFK
Dengue_virus_1_2                  TQNGRLITANPIVTDK--EKPVNIETEPPFGESYIVVGAGEKALKLSWFK
Dengue_virus_1_3                  TQNGRLITANPIVTDK--EKPVNIEAEPPFGESYIVIGAGEKALKLSWFK
Dengue_virus_1_4                  TQNGRLITANPIVTDK--EKPVNIEAEPPFGESYIVIGAGEKALKLSWFK
Dengue_virus_2_1                  YVLGRLITVNPIVTEK--DSPVNIEAEPPFGDSYIIIGVEPGQLKLNWFK
Dengue_virus_2_2                  HVLGRLITVNPIVTEK--DSPVNIEAEPPFGDSYIIIGVEPGQLKLNWFK
Dengue_virus_2_3                  HVLGRLITVNPIVTEK--DSPVNIEAEPPFGDSYIIIGVEPGQLKLNWFK
Dengue_virus_2_4                  HVLGRLITVNPIVTEK--DSPVNIEAEPPFGDSYIIIGVEPGQLKLNWFK
Dengue_virus_3_1                  AHNGRLITANPVVTKK--EEPVNIEAEPPFGESNIIIGIGDKALKINWYK
Dengue_virus_3_2                  AHNGRLITANPVVTKK--EEPVNIEAEPPFGESNIVIGIGDKALKINWYK
Dengue_virus_3_3                  AHNGRLITANPVVTKK--EEPVNIEAEPPFGESNIVIGIGDNALKINWYK
Dengue_virus_3_4                  AHNGRLITANPVVTKK--EEPVNIEAEPPFGESNIIIGIGDKALKINWYK
Dengue_virus_4_1                  KVVGRVISATPLAENT--NSVTNIELEPPFGDSYIVIGVGNSALTLHWFR
Dengue_virus_4_2                  KVIGRVISSIPLAENT--NSVTNIELEPPFGDSYIVIGVGNSALTLHWFR
Dengue_virus_4_3                  KVVGRIISSTPFAENT--NSVTNIELEPPFGDSYIVIGVGDSALTLHWFR
Dengue_virus_4_4                  KVVGRIISSTPFAENT--NSVTNIELEPPFGDSYIVIGVGDSALTLHWFR
Japanese_encephalitis_virus_1     TPVGRLVTVNPFVATSSANSKVLVEMEPPFG--------------------
Japanese_encephalitis_virus_2     TPVGRLVTVNPFVATSSANSKVLVEMEPPFGDSYIVVGRGDKQINHHWHK
Japanese_encephalitis_virus_3     TPVGRLVTVNPFVATSSSNSKVLVEMEPPFGDSYIVVGRGDKQINHHWHK
Japanese_encephalitis_virus_4     TPVGRLVTVNPFVATSSSNSKVLVEMEPPFGDSYIVVGRGDKQINHHWHK
West_Nile_virus_1                 TPVGRLVTVNPFVSVSTANAKVLIELEPPFGDSYIVVGRGEQQINHHWHK
West_Nile_virus_2                 TPVGRLVTVNPFVSVATANSKVLIELEPPFGDSYIVVGRGEQQINHHWHK
West_Nile_virus_3                 TPVGRLVTVNPFVSVATANAKVLIELEPPFGDSYIVVGRGEQQINHHWHK
West_Nile_virus_4                 TPVGRLVTVNPFVSVATANAKVLIELEPPFGDSYIVVGRGEQQINHHWHK
Yellow_fever_virus_1              VNKGILVTVNPIASTN--DDEVLIEVNPPFGDSYIIVGTGDSRLTYQWHK
Yellow_fever_virus_2              VNKGILVTVNPIASTN--DDEVLIEVNPPFGDSYIIVGTGDSRLTYQWHK
Yellow_fever_virus_3              VNKGILVTVNPIASTN--DDEVLIEVNPPFGDSYIIVGTGDSRLTYQWHK
Yellow_fever_virus_4              VNKGILVTVNPIASTN--DDEVLIEVNPPFGDSYIIVGTGDSRLTYQWHK
#=GC RF                           xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
```

```
Zika_virus_1                      sgstigkafeatvrgakrmavlgdtawdfgsvggvfnslgkgihqifgaa
Zika_virus_2                      sgstigkafeatvrgakrmavlgdtawdfgsvggvfnslgkgihqifgaa
Zika_virus_3                      sgstigkafeatvrgakrmavlgdtawdfgsvggvfnslgkgihqifgaa
Zika_virus_4                      sgstigkafeatvrgakrmavlgdtawdfgsvggvfnslgkgihqifgaa
Dengue_virus_1_1                  kgssigkmfeatargarrmailgdtawdfgsiggvftsvgklvhqifgta
Dengue_virus_1_2                  kgssigkmfeatargarrmailgdtawdfgsiggvftsvgklvhqvfgta
Dengue_virus_1_3                  kgssigkmfeatargarrmailgdtawdfgsiggvftsvgklvhqifgta
Dengue_virus_1_4                  kgssigkmfeatargarrmailgdtawdfgsiggvftsvgklvhqifgta
Dengue_virus_2_1                  kgssigqmfettmrgakrmailgdtawdfgslggvftsvgkalhqvfgai
Dengue_virus_2_2                  kgssigqmfettmrgakrmailgdtawdfgslggvftsigkalhqvfgai
Dengue_virus_2_3                  kgssigqmfettmrgakrmailgdtawdfgslggvftsigkalhqvfgai
Dengue_virus_2_4                  kgssigqmfettmrgakrmailgdtawdfgslggvftsigkalhqvfgai
Dengue_virus_3_1                  kgssigkmfeatargarrmailgdtawdfgsvggvlnslgkmvhqifgsa
Dengue_virus_3_2                  kgssigkmfeatargarrmailgdtawdfgsvggvlnslgkmvhqifgsa
Dengue_virus_3_3                  kgssigkmfeatargarrmailgdtawdfgsvggvlnslgkmvhqifgsa
Dengue_virus_3_4                  kgssigkmfeatargarrmailgdtawdfgsvggvlnslgkmvhqifgsa
Dengue_virus_4_1                  kgssigkmfestyrgakrmailgetawdfgsvgglftslgkavhqvfgsv
Dengue_virus_4_2                  kgssigkmfestyrgakrmailgetawdfgsvgglftslgkavhqvfgsv
Dengue_virus_4_3                  kgssigkmfestyrgakrmailgetawdfgsvgglftslgkavhqvfgsv
Dengue_virus_4_4                  kgssigkmfestyrgakrmailgetawdfgsvgglftslgkavhqvfgsv
Japanese_encephalitis_virus_1     ............................................
Japanese_encephalitis_virus_2     agstlgkafsttlkgaqrlaalgdtawdfgsiggvfnsigkavhqvfgga
Japanese_encephalitis_virus_3     agstlgkafsttlkgaqrlaalgdtawdfgsiggvfnsigkavhqvfgg.
Japanese_encephalitis_virus_4     agstlgkafsttlkgaqrlaalgdtawdfgsiggvfnsigkavhqvfgg.
West_Nile_virus_1                 sgssigkaftttlkgaqrlaalgdtawdfgsvggvftsvgkavhqvfgga
West_Nile_virus_2                 sgssigkaftttlrgaqrlaalgdtawdfgsvggvfasvgkaihqvfgga
West_Nile_virus_3                 sgssigkaftttlkgaqrlaalgdtawdfgsvggvftsvgkavhqvfgga
West_Nile_virus_4                 sgssigkaftttlkgaqrlaalgdtawdfgsvggvftsvgkavhqvfgga
Yellow_fever_virus_1              egssigklftqtmkgaerlavmgdaawdfgsaggfftsvgkgihtvfgsa
Yellow_fever_virus_2              egssigklftqtmkgaerlavmgdaawdfgsaggfftsvgkgihtvfgsa
Yellow_fever_virus_3              egssigklftqtmkgaerlavmgdaawdfgsaggfftsvgkgihtvfgsa
Yellow_fever_virus_4              egssigklftqtmkgaerlavmgdaawdfgsaggfftsvgkgihtvfgsa
#=GC RF                           ............................................
```

Flavivirus glycoprotein, immunoglobulin-like domain

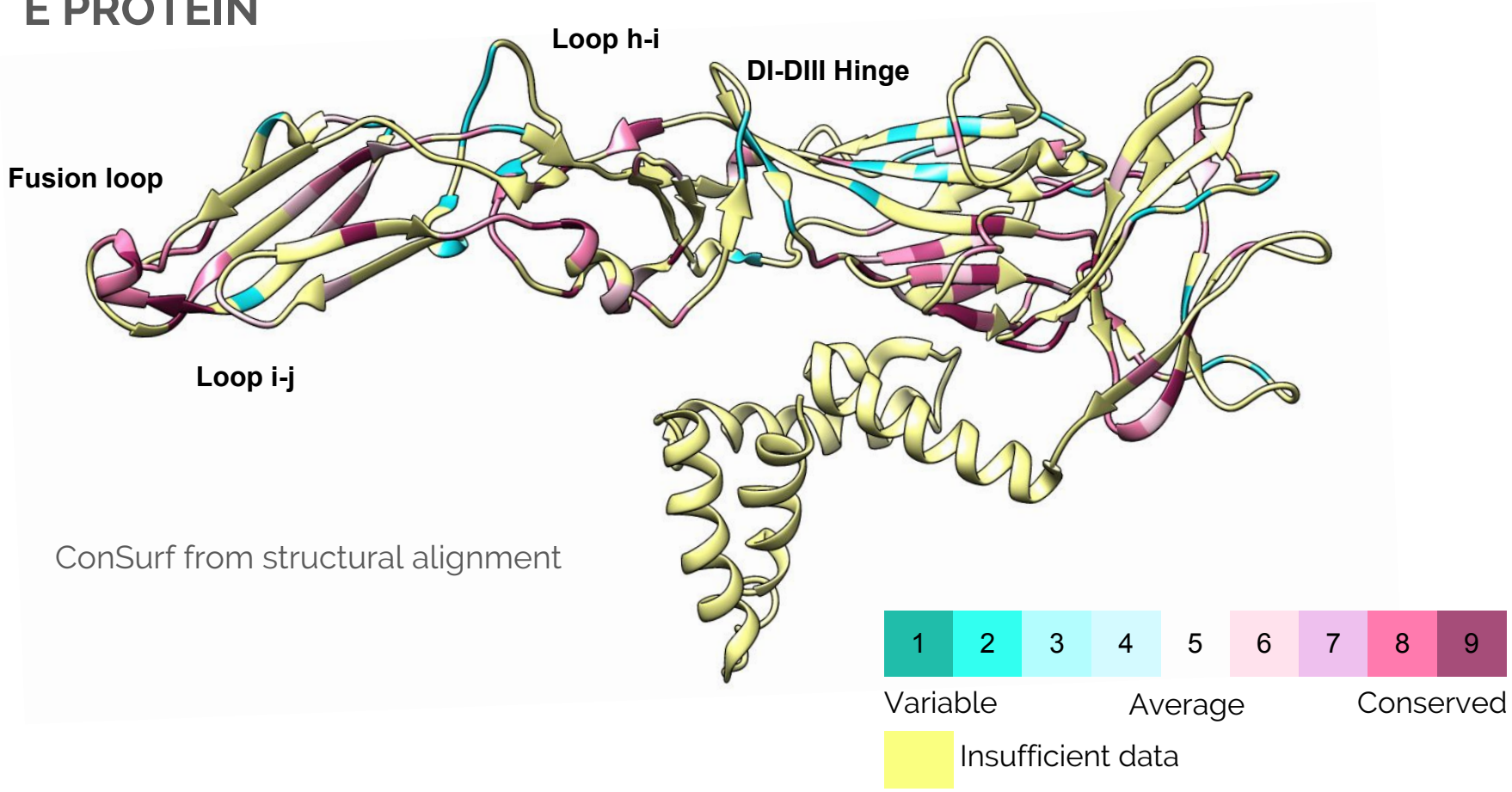
ANNEX

HMM alignment

```
Zika_virus_1                        fkslfggmswfsqiligtllvwlglntkngsisltclalggvmiflstav  Zika_virus_1                        sa
Zika_virus_2                        fkslfggmswfsqiligtllvwlglntkngsisltclalggvmiflstav  Zika_virus_2                        sa
Zika_virus_3                        fkslfggmswfsqiligtllvwlglntkngsisltclalggvmiflstav  Zika_virus_3                        sa
Zika_virus_4                        fkslfggmswfsqiligtllvwlglntkngsisltclalggvmiflstav  Zika_virus_4                        sa
Dengue_virus_1_1                    ygvlfsgvswtmkigigvlltwlglnsrstslsmtciavglvtlylgvmv  Dengue_virus_1_1                    qa
Dengue_virus_1_2                    ygvlfsgvswtmkigigilltwlglnsrstslsmtciavgmvtlylgvmv  Dengue_virus_1_2                    qa
Dengue_virus_1_3                    ygvlfsgvswtmkigigvlltwlglnsrstsfsmtciavglvtlylgvmv  Dengue_virus_1_3                    qa
Dengue_virus_1_4                    ygvlfsgvswtmkigigvlltwlglnsrstslsmtciavglvtlylgvmv  Dengue_virus_1_4                    qa
Dengue_virus_2_1                    ygaafsgvswtmkiligviitwigmnsrstslsvslvlvgivtlylgvmv  Dengue_virus_2_1                    qa
Dengue_virus_2_2                    ygaafsgvswtmkiligviitwigmnsrstslsvslvlvgvvtlylgamv  Dengue_virus_2_2                    qa
Dengue_virus_2_3                    ygaafsgvswtmkiligviitwigmnsrstslsvslvlvgvvtlylgavv  Dengue_virus_2_3                    qa
Dengue_virus_2_4                    ygaafsgvswtmkiligviitwigmnsrstslsvslvlvgvvtlylgvmv  Dengue_virus_2_4                    qa
Dengue_virus_3_1                    ytalfsgvswimkigigvlltwiglnskntsmsfscivigiitlylgavv  Dengue_virus_3_1                    qa
Dengue_virus_3_2                    ytalfggvswimkigigvlltwiglnskntsmsfscivigiitlylgtvv  Dengue_virus_3_2                    qa
Dengue_virus_3_3                    ytalfsgvswvmkigigvlltwiglnskntsmsfsciaigiitlylgavv  Dengue_virus_3_3                    qa
Dengue_virus_3_4                    ytalfsgvswimkigigvlltwiglnskntsmsfscivigiitlylgavv  Dengue_virus_3_4                    qa
Dengue_virus_4_1                    yttmfggvswmiriligflvlwigtnsrntsmamtciavggitlflgftv  Dengue_virus_4_1                    qa
Dengue_virus_4_2                    yttmfggvswmiriligflvlwigtnsrntsmamtciavggitlflgftv  Dengue_virus_4_2                    qa
Dengue_virus_4_3                    yttmfggvswmvriligflvlwigtnsrntsmamtciavggitlflgftv  Dengue_virus_4_3                    qa
Dengue_virus_4_4                    yttmfggvswmvriligflvlwigtnsrntsmamtciavggitlflgftv  Dengue_virus_4_4                    qa
Japanese_encephalitis_virus_1       ...............................................  Japanese_encephalitis_virus_1       ..
Japanese_encephalitis_virus_2       frtlfggmswitqglmgalllwmgvnardrsialaflatggvlvflatnv  Japanese_encephalitis_virus_2       ha
Japanese_encephalitis_virus_3       ...............................................  Japanese_encephalitis_virus_3       ..
Japanese_encephalitis_virus_4       ...............................................  Japanese_encephalitis_virus_4       ..
West_Nile_virus_1                   frslfggmswitqgllgalllwmginardrsialtflavggvllflsvnv  West_Nile_virus_1                   ha
West_Nile_virus_2                   frslfggmswitqgllgalllwmginardrsiamtflavggvllflsvnv  West_Nile_virus_2                   ha
West_Nile_virus_3                   frslfggmswitqgllgalllwmginardrsialtflavggvllflsvnv  West_Nile_virus_3                   ha
West_Nile_virus_4                   frslfggmswitqgllgalllwmginardrsialtflavggvllflsvnv  West_Nile_virus_4                   ha
Yellow_fever_virus_1                fqglfgglswitkvimgvvliwvgintrnmtmsmsmilvgvimmflslgv  Yellow_fever_virus_1                ga
Yellow_fever_virus_2                fqglfgglswitkvimgvvliwvgintrnmtmsmsmilvgvimmflslgv  Yellow_fever_virus_2                ga
Yellow_fever_virus_3                fqglfgglswitkvimgvvliwvgintrnmtmsmsmilvgvimmflslgv  Yellow_fever_virus_3                ga
Yellow_fever_virus_4                fqglfgglswitkvimgvvliwvgintrnmtmsmsmilvgvimmflslgv  Yellow_fever_virus_4                ga
#=GC RF                             ...............................................  #=GC RF                             ..
                                                                                        //
```

ANNEX

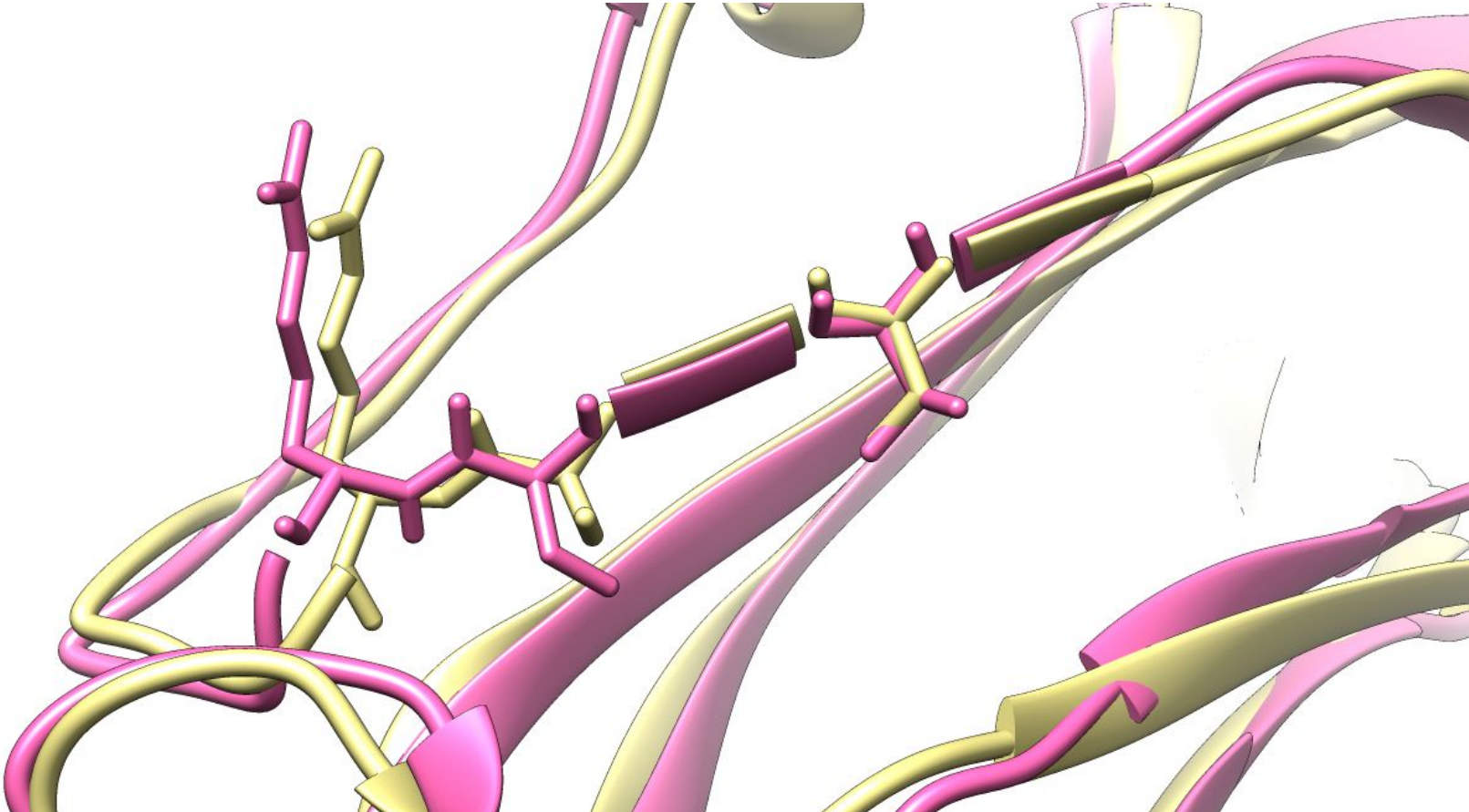
E PROTEIN



ANNEX

Cross-neutralizing

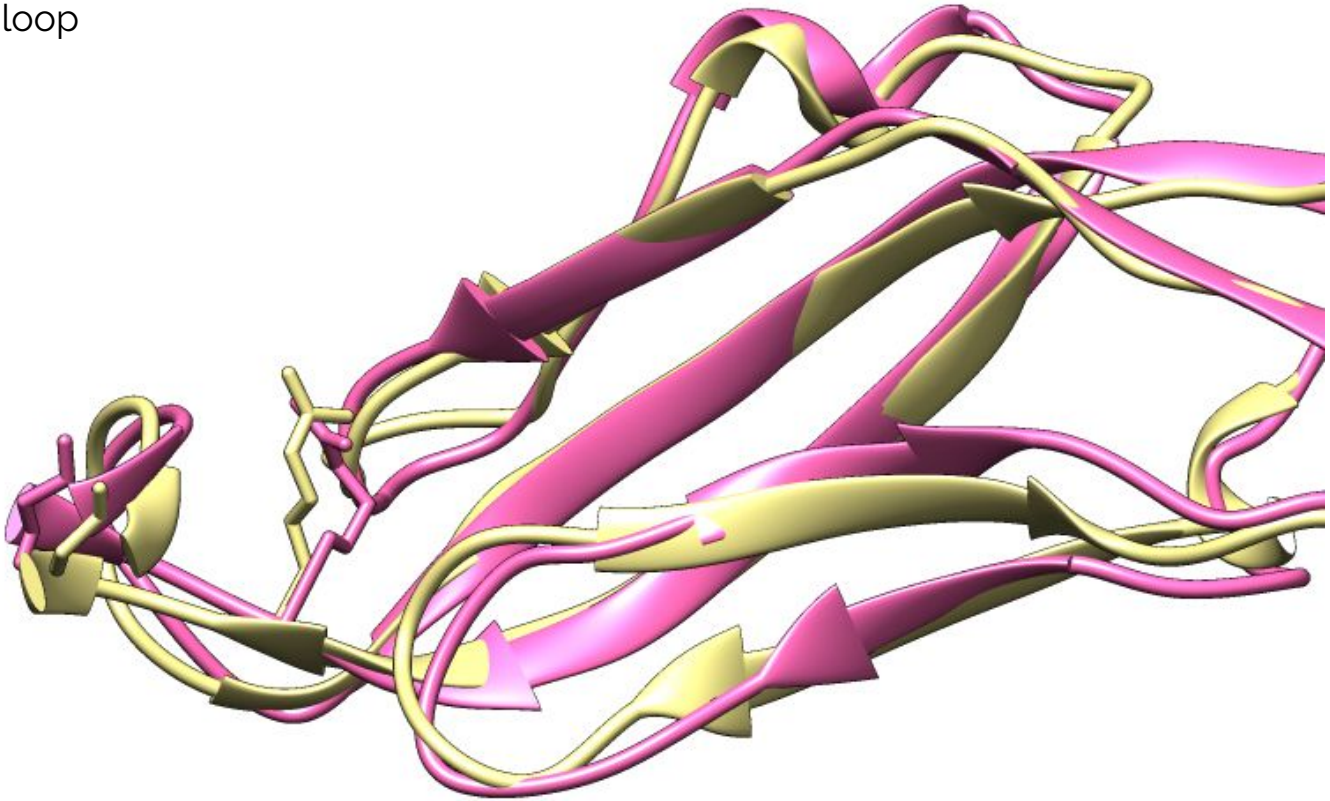
b strand



ANNEX

Cross-neutralizing

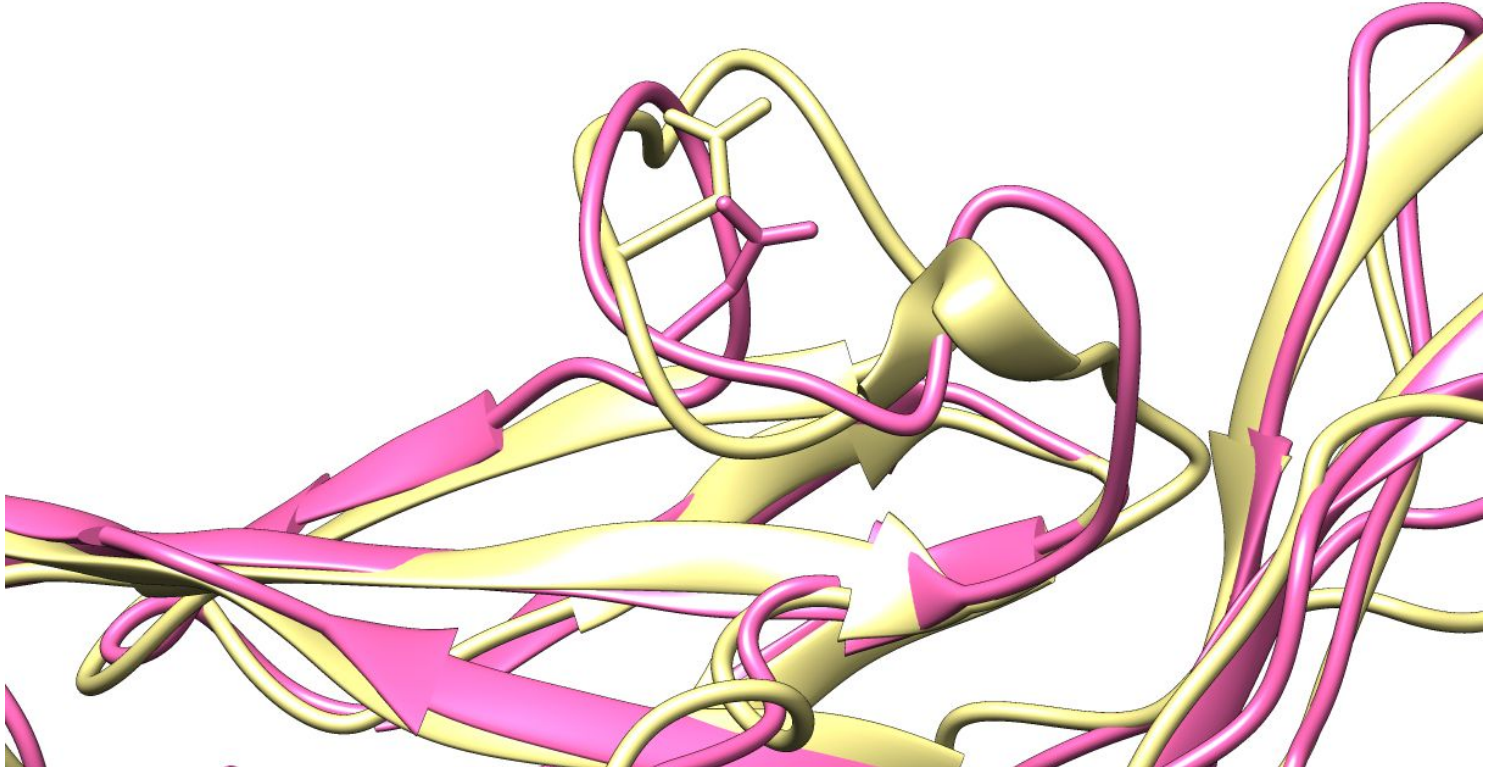
Fusion loop



ANNEX

Cross-neutralizing

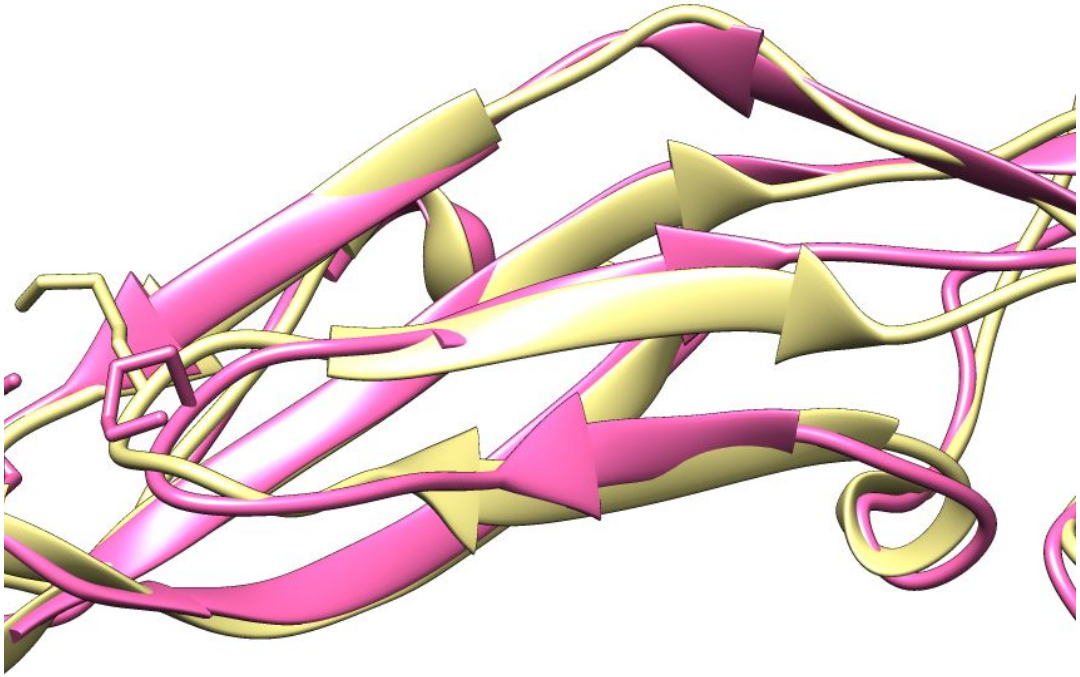
Glycosylation loop



ANNEX

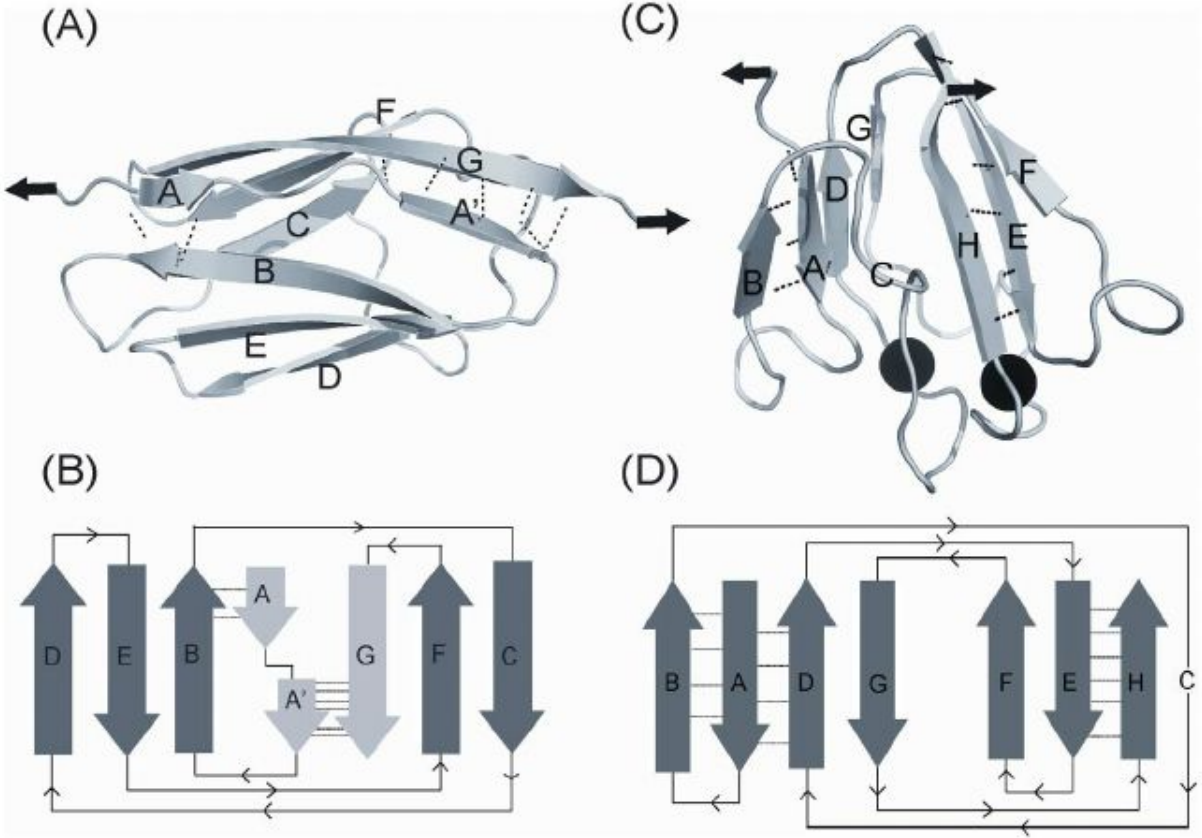
Cross-neutralizing

i-j loop



ANNEX

Beta-sandwich topological diagram



Ramanujam, V., Kotamarthi, H. and Ainavarapu, S. (2014). Ca2+ Binding Enhanced Mechanical Stability of an Archaeal Crystallin. *PLoS ONE*, 9(4), p.e94513.