



**HAL**  
open science

## **Adnaviria: a New Realm for Archaeal Filamentous Viruses with Linear A-Form Double-Stranded DNA Genomes**

Mart Krupovic, Jens H Kuhn, Fengbin Wang, Diana P Baquero, Valerian V Dolja, Edward H Egelman, David Prangishvili, Eugene V Koonin

► **To cite this version:**

Mart Krupovic, Jens H Kuhn, Fengbin Wang, Diana P Baquero, Valerian V Dolja, et al.. Adnaviria: a New Realm for Archaeal Filamentous Viruses with Linear A-Form Double-Stranded DNA Genomes. Journal of Virology, 2021, 95 (15), pp.e00673-21. 10.1128/JVI.00673-21 . pasteur-03284792

**HAL Id: pasteur-03284792**

**<https://pasteur.hal.science/pasteur-03284792>**

Submitted on 12 Jul 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 ***Adnaviria*: a new realm for archaeal filamentous viruses with linear A-form double-**  
2 **stranded DNA genomes**

3

4 Mart Krupovic<sup>a\*</sup>, Jens H. Kuhn<sup>b</sup>, Fengbin Wang<sup>c</sup>, Diana P. Baquero<sup>a</sup>, Valerian V. Dolja<sup>d</sup>,  
5 Edward H. Egelman<sup>c</sup>, David Prangishvili<sup>a,c</sup>, Eugene V. Koonin<sup>f</sup>

6

7 <sup>a</sup>Archaeal Virology Unit, Institut Pasteur, Paris, France

8 <sup>b</sup>NIH/NIAID/DCR/Integrated Research Facility at Fort Detrick (IRF-Frederick), Fort Detrick,  
9 Frederick, MD, USA

10 <sup>c</sup>Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville,  
11 VA, USA

12 <sup>d</sup>Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, USA

13 <sup>e</sup>Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia

14 <sup>f</sup>National Center for Biotechnology Information, National Library of Medicine, National  
15 Institutes of Health, Bethesda, MD, USA

16

17 \*Correspondence to Mart Krupovic, mart.krupovic@pasteur.fr

18

19 Keywords: A-form DNA, *Ligamenvirales*, *Tokiviricetes*, *Rudiviridae*, *Lipothrixviridae*,

20 *Tristromaviridae*, hyperthermophilic archaea, virus evolution, virus classification, virus

21 taxonomy, International Committee on Taxonomy of Viruses (ICTV), major capsid protein, virus

22 structure and assembly

23

24 **Abstract**

25 **The International Committee on Taxonomy of Viruses (ICTV) has recently adopted a**  
26 **comprehensive, hierarchical system of virus taxa. The highest rank in this hierarchy are**  
27 **realms, each of which is considered monophyletic but apparently originated independently**  
28 **of other realms. Here we announce the creation of a new realm, *Adnaviria*, which unifies**  
29 **archaeal filamentous viruses with linear A-form double-stranded DNA genomes and**  
30 **characteristic major capsid proteins unrelated to those encoded by other known viruses.**

31

32 **Text**

33 In 2018, the International Committee on Taxonomy of Viruses (ICTV) expanded the number of  
34 taxonomic ranks available for virus classification from 5 to 15 (1, 2). This development enabled  
35 formal recognition of the evolutionary kinships among distantly related viruses and led to  
36 creation of four realms—virus taxa that are roughly equivalent to the domain rank in cellular  
37 taxonomy (3): The realm *Riboviria* encompasses all RNA viruses and reverse-transcribing  
38 viruses that encode homologous RNA-directed RNA polymerases and reverse transcriptases,  
39 respectively; *Monodnaviria* comprises viruses with predominantly single-stranded (ss) DNA  
40 genomes and encoding rolling-circle replication initiation endonucleases of the HUH  
41 superfamily; *Duplodnaviria* unifies viruses with double-stranded DNA genomes that produce  
42 virions with icosahedral capsids formed by major capsid proteins (MCPs) with the HK97 fold;  
43 and *Varidnaviria* comprises dsDNA viruses with icosahedral capsids built from MCPs with  
44 double-jelly roll fold (4). However, many virus families remained unassigned to higher taxa due  
45 to the lack of demonstrable evolutionary relationships to viruses assigned to the four realms.

46 Here we announce the creation, and recent official acceptance by the ICTV  
47 (<https://talk.ictvonline.org/taxonomy/>), of a new realm, *Adnaviria*, which encompasses  
48 structurally related archaeal filamentous viruses with dsDNA genomes that adopt the A-form  
49 conformation within their virions.

50 Filamentous viruses infect hosts of all cellular domains but, despite overall similar morphology,  
51 virions of eukaryotic, bacterial, and archaeal filamentous viruses are built from capsid proteins  
52 with different structural folds (5). Furthermore, the types of the viral genomes that are protected  
53 by the corresponding capsid proteins are radically different among viruses infecting hosts from  
54 the different domains of life (6-11): All known eukaryotic filamentous viruses have linear ssRNA  
55 genomes and are classified in realm *Riboviria* (4); all known bacterial filamentous viruses have  
56 circular ssDNA genomes and belong to realm *Monodnaviria* (4, 12); and all known archaeal  
57 filamentous viruses have dsDNA genomes and, until recently, have remained unassigned to taxa  
58 ranked higher than order.

59 Archaeal viruses that form filamentous virions are currently classified into four families:  
60 *Clavaviridae*, *Lipothrixviridae*, *Rudiviridae*, and *Tristromaviridae* (13). Based on the shared  
61 gene content, structural similarity of their virions, and homology of the MCPs, families  
62 *Lipothrixviridae* and *Rudiviridae* were included in order *Ligamenvirales* (14). In virions of both  
63 families, the nucleoprotein helix is composed of asymmetric units containing two MCP  
64 molecules, a homodimer in the case of rudivirids and a heterodimer of paralogous MCPs in the  
65 case of lipothrixvirids (Figure 1) (11, 15). The MCPs of ligamenviral particles have a unique  $\alpha$ -  
66 helical fold first found in the MCP of rudivirid *Sulfolobus islandicus* rod-shaped virus 2 (SIRV2)  
67 (16). Lipothrixvirids and rudivirids share a characteristic feature in that the interaction between

68 the MCP dimer and the linear dsDNA genome maintains the DNA in the A form. Consequently,  
69 the entire genome adopts the A-form in virions (15-18).

70 Clavavirids (19, 20) and tristromavirids (21) do not encode proteins with recognizable sequence  
71 similarity to proteins of ligamenvirals (22). Hence, the two families were not included in  
72 *Ligamenvirales*. Recently, the structure of particles produced by tristromavirid *Pyrobaculum*  
73 filamentous virus 2 (PFV2) has been characterized by cryogenic electron microscopy (cryo-EM)  
74 (23). Unexpectedly, the two nucleocapsid proteins of tristromavirids were found to be  
75 structurally related to those of ligamenvirals, and the virion organizations were discovered to be  
76 remarkably similar, including the A-form conformation of the genomic dsDNA (23) (Figure 1).  
77 Similar to lipothrixvirids, virions of tristromavirids are enveloped by a lipid membrane (24).  
78 Based on the structural similarity between members of *Ligamenvirales* and *Tristromaviridae*, we  
79 inferred that they share a common origin (23). To formalize this evolutionary relationship,  
80 *Tristromaviridae* was assigned to a new order, *Primavirales*, which, along with *Ligamenvirales*,  
81 was included in a new class, *Tokiviricetes*.

82 Members of the *Tokiviricetes* represent one of the most commonly detected virus groups in  
83 terrestrial hot springs and are globally distributed (25-27). Rudivirids have been extensively  
84 studied in particular, and many aspects of their life cycles are understood in considerable detail  
85 (28). Notably, the mechanisms of genome replication do not appear to be conserved among  
86 tokiviricetes. It has been proposed that rudivirus SIRV2 uses a combination of strand-  
87 displacement, rolling-circle, and strand-coupled genome replication mechanisms, which generate  
88 multimeric, highly branched, “brush-like” intermediates reaching lengths of >1,200 kb (~34  
89 genome units) (29). The latter are then processed into unit-length, linear genomes with  
90 covalently linked hairpin ends. The genome replication of the lipothrixvirid *Acidianus*

91 filamentous virus 1 has been suggested to start by the formation of a D loop and to progress by  
92 the strand displacement replication mechanism, whereas termination relies on recombination  
93 events through the formation of terminal loop-like structures, though the genes involved in this  
94 unique mechanism of replication remain unknown (30). Similar to rudivirid genomes,  
95 tristromavirid genomes have terminal inverted repeats (21), but the replication mechanism of  
96 these viruses has not been investigated.

97 High-resolution structures are now available for virions of six distinct tokiviricetes (11, 15). Like  
98 many structurally related viruses in the two other realms of dsDNA viruses (*Duplodnaviria* and  
99 *Varidnaviria*), there is no detectable sequence similarity among the capsid proteins of viruses  
100 from different tokiviricete families, suggesting a vast undescribed diversity of viruses in this part  
101 of the virosphere. Indeed, it has been suggested that tokiviricetes were present in the last archaeal  
102 common ancestor, and possibly even in the last universal cellular ancestor (LUCA) (31).  
103 Regardless, the available data unequivocally show that archaeal filamentous viruses do not fall  
104 into any of the four established realms. Thus, given the lack of detectable relationship with other  
105 viruses and officially acknowledging the uniqueness of the three families of archaeal filamentous  
106 viruses that contain A-form DNA in their virions, a new taxon of the highest rank, realm  
107 *Adnaviria*, was created for their classification. To bridge the gap between the class and realm  
108 taxa, intermediate kingdom and phylum taxa, named *Zilligvirae* and *Taleaviricota*, respectively,  
109 were established.

110 In contrast, structural characterization of clavavirid *Aeropyrum pernix* bacilliform virus 1  
111 (APBV1) particles by cryo-EM confirmed that the fold of its MCP and its overall virion  
112 organization are unrelated to that of ligamenviral, tristromavirid, or other characterized virus

113 particles (32). Therefore, notwithstanding their filamentous virions, clavavirids are not included  
114 in realm *Adnaviria* and likely represent another realm to be established in the future.

115

116 **Etymology of new taxa recently accepted by the ICTV:**

- 117 • *Adnaviria*; from A-form DNA characteristic of viruses in this realm and suffix *-viria* for  
118 realm taxa.
- 119 • *Zilligvirae*; after Wolfram Zillig (1925–2005), a pioneer of research on  
120 hyperthermophilic archaeal viruses, and the suffix *-virae* for kingdom taxa.
- 121 • *Taleaviricota*; from Latin *talea*, meaning “rod” (referring to the virion morphology), and  
122 suffix *-viricota* for phylum taxa.
- 123 • *Tokiviricetes*; from Georgian თოკო (*toki*), meaning “thread”, and suffix *-viricetes* for  
124 class taxa.
- 125 • *Primavirales*; from Latin *prima*, meaning “first”, referring to the fact that Thermoproteus  
126 tenax virus 1, classified in this order, was the first hyperthermophilic archaeal virus to be  
127 isolated in 1983 (33).

128

129 **ACKNOWLEDGMENTS**

130 We are grateful to Anya Crane (National Institutes of Health [NIH] National Institute of Allergy  
131 and Infectious Diseases [NIAID] Division of Clinical Research [DCR] Integrated Research  
132 Facility at Fort Detrick [IRF-Frederick], Frederick, MD, USA) for editing the manuscript.

133 M.K. was supported by l'Agence Nationale de la Recherche (grant ANR-20-CE20-0009-02) and  
134 Emergence(s) project MEMREMA from Ville de Paris. E.V.K. is supported by the Intramural  
135 Research Program of the National Institutes of Health of the USA (National Library of  
136 Medicine). E.H.E. was supported by NIH R35GM122510. This work was supported in part  
137 through Laulima Government Solutions, LLC prime contract with the US National Institute of  
138 Allergy and Infectious Diseases (NIAID) under Contract No. HHSN272201800013C. J.H.K.  
139 performed this work as an employee of Tunnell Government Services (TGS), a subcontractor of  
140 Laulima Government Solutions, LLC under Contract No. HHSN272201800013C.

141 The views and conclusions contained in this document are those of the authors and should not be  
142 interpreted as necessarily representing the official policies, either expressed or implied, of the US  
143 Department of Health and Human Services or of the institutions and companies affiliated with  
144 the authors.

145

146 **Figure legend**

147 **Figure 1. Taxonomic organization of realm *Adnaviria*.** **A.** Taxonomic ranks from family to  
148 realm are rendered with different colors. **B-D.** Cryogenic electron microscopy shows structures  
149 of the major capsid protein (MCP) homodimer (characteristic of rudivirids) and heterodimer  
150 (characteristic of lipothrixvirids and tristromavirids) (**B**), MCPs bound to viral double-stranded  
151 (ds) DNA genome in A-form conformation (**C**), and side views of the viral nucleocapsid are  
152 shown for each constituent family (**D**). *Rudiviridae*, *Lipothrixviridae*, and *Tristromaviridae* are  
153 represented by structures of *Sulfolobus islandicus* rod-shaped virus 2 (PDB entry: 3J9X),  
154 *Acidianus filamentous virus 1* (PDB entry: 5WTG), and *Pyrobaculum filamentous virus 2* (PDB  
155 entry: 6VTB), respectively. The figure is modified from ref ([11](#)).

156 **References**

- 157 1. Gorbalenya AE, Krupovic M, Mushegian A, Kropinski AM, Siddell SG, Varsani A,  
158 Adams MJ, Davison AJ, Dutilh BE, Harrach B, Harrison RL, Junglen S, King AMQ,  
159 Knowles NJ, Lefkowitz EJ, Nibert ML, Rubino L, Sabanadzovic S, Sanfaçon H,  
160 Simmonds P, Walker PJ, Zerbini FM, Kuhn JH. 2020. The new scope of virus taxonomy:  
161 partitioning the virosphere into 15 hierarchical ranks. *Nat Microbiol* 5:668-674.
- 162 2. Siddell SG, Walker PJ, Lefkowitz EJ, Mushegian AR, Adams MJ, Dutilh BE,  
163 Gorbalenya AE, Harrach Bz, Harrison RL, Junglen S, Knowles NJ, Kropinski AM,  
164 Krupovic M, Kuhn JH, Nibert M, Rubino L, Sabanadzovic S, Sanfaçon H, Simmonds P,  
165 Varsani A, Zerbini FM, Davison AJ. 2019. Additional changes to taxonomy ratified in a  
166 special vote by the International Committee on Taxonomy of Viruses (October 2018).  
167 *Arch Virol* 164:943-946.
- 168 3. Walker PJ, Siddell SG, Lefkowitz EJ, Mushegian AR, Dempsey DM, Dutilh BE, Harrach  
169 B, Harrison RL, Hendrickson RC, Junglen S, Knowles NJ, Kropinski AM, Krupovic M,  
170 Kuhn JH, Nibert M, Rubino L, Sabanadzovic S, Simmonds P, Varsani A, Zerbini FM,  
171 Davison AJ. 2019. Changes to virus taxonomy and the International Code of Virus  
172 Classification and Nomenclature ratified by the International Committee on Taxonomy of  
173 Viruses (2019). *Arch Virol* 164:2417-2429.
- 174 4. Koonin EV, Dolja VV, Krupovic M, Varsani A, Wolf YI, Yutin N, Zerbini FM, Kuhn  
175 JH. 2020. Global organization and proposed megataxonomy of the virus world. *Microbiol*  
176 *Mol Biol Rev* 84:e00061-19.
- 177 5. Krupovic M, Koonin EV. 2017. Multiple origins of viral capsid proteins from cellular  
178 ancestors. *Proc Natl Acad Sci U S A* 114:E2401-E2410.

- 179 6. DiMaio F, Chen C-C, Yu X, Frenz B, Hsu Y-H, Lin N-S, Egelman EH. 2015. The  
180 molecular basis for flexibility in the flexible filamentous plant viruses. *Nat Struct Mol*  
181 *Biol* 22:642-4.
- 182 7. Klug A. 1999. The tobacco mosaic virus particle: structure and assembly. *Philos Trans R*  
183 *Soc Lond B Biol Sci* 354:531-5.
- 184 8. Stubbs G, Kendall A. 2012. Helical viruses. *Adv Exp Med Biol* 726:631-58.
- 185 9. Tarafder AK, von Kügelgen A, Mellul AJ, Schulze U, Aarts DGAL, Bharat TAM. 2020.  
186 Phage liquid crystalline droplets form occlusive sheaths that encapsulate and protect  
187 infectious rod-shaped bacteria. *Proc Natl Acad Sci U S A* 117:4724-4731.
- 188 10. Zamora M, Méndez-López E, Agirrezabala X, Cuesta R, Lavín JL, Sánchez-Pina MA,  
189 Aranda MA, Valle M. 2017. Potyvirus virion structure shows conserved protein fold and  
190 RNA binding site in ssRNA viruses. *Sci Adv* 3:eaa02182.
- 191 11. Baquero DP, Liu Y, Wang F, Egelman EH, Prangishvili D, Krupovic M. 2020. Structure  
192 and assembly of archaeal viruses. *Adv Virus Res* 108:127-164.
- 193 12. Roux S, Krupovic M, Daly RA, Borges AL, Nayfach S, Schulz F, Sharrar A, Matheus  
194 Carnevali PB, Cheng J-F, Ivanova NN, Bondy-Denomy J, Wrighton KC, Woyke T, Visel  
195 A, Kyrpides NC, Eloe-Fadrosh EA. 2019. Cryptic inoviruses revealed as pervasive in  
196 bacteria and archaea across Earth's biomes. *Nat Microbiol* 4:1895-1906.
- 197 13. Prangishvili D, Bamford DH, Forterre P, Iranzo J, Koonin EV, Krupovic M. 2017. The  
198 enigmatic archaeal virosphere. *Nat Rev Microbiol* 15:724-739.
- 199 14. Prangishvili D, Krupovic M. 2012. A new proposed taxon for double-stranded DNA  
200 viruses, the order "Ligamenvirales". *Arch Virol* 157:791-5.

- 201 15. Wang F, Baquero DP, Beltran LC, Su Z, Osinski T, Zheng W, Prangishvili D, Krupovic  
202 M, Egelman EH. 2020. Structures of filamentous viruses infecting hyperthermophilic  
203 archaea explain DNA stabilization in extreme environments. *Proc Natl Acad Sci U S A*  
204 117:19643-19652.
- 205 16. DiMaio F, Yu X, Rensen E, Krupovic M, Prangishvili D, Egelman EH. 2015. A virus that  
206 infects a hyperthermophile encapsidates A-form DNA. *Science* 348:914-7.
- 207 17. Kasson P, DiMaio F, Yu X, Lucas-Staat S, Krupovic M, Schouten S, Prangishvili D,  
208 Egelman EH. 2017. Model for a novel membrane envelope in a filamentous  
209 hyperthermophilic virus. *Elife* 6:e26268.
- 210 18. Liu Y, Osinski T, Wang F, Krupovic M, Schouten S, Kasson P, Prangishvili D, Egelman  
211 EH. 2018. Structural conservation in a membrane-enveloped filamentous virus infecting a  
212 hyperthermophilic acidophile. *Nat Commun* 9:3360.
- 213 19. Prangishvili D, Mochizuki T, Liu Y, Krupovic M, ICTV Report Consortium. 2019. ICTV  
214 Virus Taxonomy Profile: *Clavaviridae*. *J Gen Virol* 100:1267-1268.
- 215 20. Mochizuki T, Yoshida T, Tanaka R, Forterre P, Sako Y, Prangishvili D. 2010. Diversity  
216 of viruses of the hyperthermophilic archaeal genus *Aeropyrum*, and isolation of the  
217 *Aeropyrum pernix* bacilliform virus 1, APBV1, the first representative of the family  
218 *Clavaviridae*. *Virology* 402:347-54.
- 219 21. Prangishvili D, Rensen E, Mochizuki T, Krupovic M, ICTV Report Consortium. 2019.  
220 ICTV Virus Taxonomy Profile: *Tristromaviridae*. *J Gen Virol* 100:135-136.
- 221 22. Iranzo J, Koonin EV, Prangishvili D, Krupovic M. 2016. Bipartite network analysis of  
222 the archaeal virosphere: evolutionary connections between viruses and capsidless mobile  
223 elements. *J Virol* 90:11043-11055.

- 224 23. Wang F, Baquero DP, Su Z, Osinski T, Prangishvili D, Egelman EH, Krupovic M. 2020.  
225 Structure of a filamentous virus uncovers familial ties within the archaeal virosphere.  
226 *Virus Evol* 6:veaa023.
- 227 24. Rensen EI, Mochizuki T, Quemin E, Schouten S, Krupovic M, Prangishvili D. 2016. A  
228 virus of hyperthermophilic archaea with a unique architecture among DNA viruses. *Proc*  
229 *Natl Acad Sci U S A* 113:2478-83.
- 230 25. Baquero DP, Contursi P, Piochi M, Bartolucci S, Liu Y, Cvirkaite-Krupovic V,  
231 Prangishvili D, Krupovic M. 2020. New virus isolates from Italian hydrothermal  
232 environments underscore the biogeographic pattern in archaeal virus communities. *ISME*  
233 *J* 14:1821-1833.
- 234 26. Bautista MA, Black JA, Youngblut ND, Whitaker RJ. 2017. Differentiation and structure  
235 in *Sulfolobus islandicus* rod-shaped virus populations. *Viruses* 9:120.
- 236 27. Prangishvili D, Arnold HP, Gotz D, Ziese U, Holz I, Kristjansson JK, Zillig W. 1999. A  
237 novel virus family, the *Rudiviridae*: Structure, virus-host interactions and genome  
238 variability of the *sulfolobus* viruses SIRV1 and SIRV2. *Genetics* 152:1387-96.
- 239 28. Prangishvili D, Koonin EV, Krupovic M. 2013. Genomics and biology of rudiviruses, a  
240 model for the study of virus-host interactions in Archaea. *Biochem Soc Trans* 41:443-50.
- 241 29. Martínez-Alvarez L, Bell SD, Peng X. 2016. Multiple consecutive initiation of  
242 replication producing novel brush-like intermediates at the termini of linear viral dsDNA  
243 genomes with hairpin ends. *Nucleic Acids Res* 44:8799-8809.
- 244 30. Pina M, Basta T, Quax TE, Joubert A, Baconnais S, Cortez D, Lambert S, Le Cam E,  
245 Bell SD, Forterre P, Prangishvili D. 2014. Unique genome replication mechanism of the  
246 archaeal virus AFV1. *Mol Microbiol* 92:1313-25.

- 247 31. Krupovic M, Dolja VV, Koonin EV. 2020. The LUCA and its complex virome. Nat Rev  
248 Microbiol 18:661-670.
- 249 32. Pchelkine D, Gillum A, Mochizuki T, Lucas-Staat S, Liu Y, Krupovic M, Phillips SEV,  
250 Prangishvili D, Huiskonen JT. 2017. Unique architecture of thermophilic archaeal virus  
251 APBV1 and its genome packaging. Nat Commun 8:1436.
- 252 33. Janekovic D, Wunderl S, Holz I, Zillig W, Gierl A, Neumann H. 1983. TTV1, TTV2 and  
253 TTV3, a family of viruses of the extremely thermophilic, anaerobic, sulfur reducing  
254 archaeobacterium *Thermoproteus tenax*. Mol Gen Genet 192:39-45.
- 255

A

Realm:

*Adnaviria*

Kingdom:

*Zilligvirae*

Phylum:

*Taleaviricota*

Class:

*Tokiviricetes*

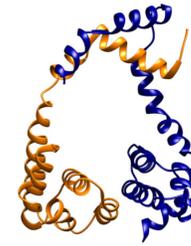
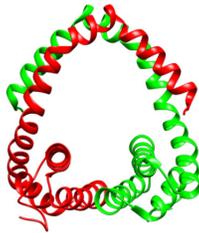
Order:

*Ligamenvirales**Primavirales*

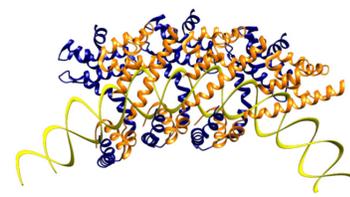
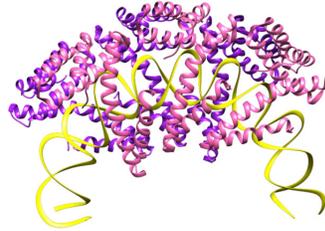
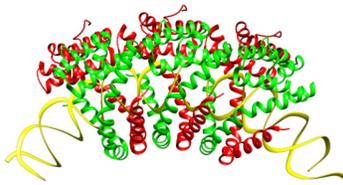
Family:

*Rudiviridae**Lipothrixviridae**Tristromaviridae*

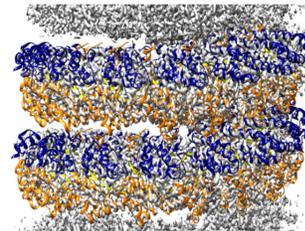
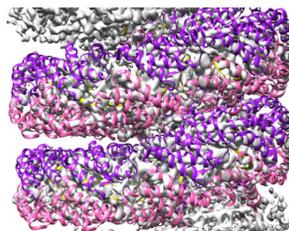
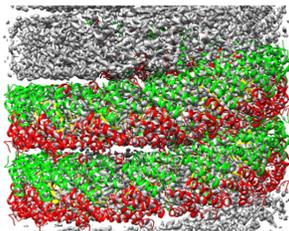
B

MCP  
dimer

C

3 MCP  
dimers  
bound to  
A-DNA

D

virion  
superhelix  
(side view)