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Chapter 497

# Arterivirus nsp2 Cysteine Proteinase

## Databanks

*MEROPS name*: equine arteritis virus papain-like peptidase 2-type cysteine peptidase
*MEROPS classification*: clan unassigned, family C33, peptidase C33.001
*Species distribution*: known only from equine arteritis virus
*Reference sequence from*: equine arteritis virus (UniProt: P19811)

## Name and History

The family *Arteriviridae* currently includes the genetically distinct members equine arteritis virus (EAV; the family prototype), porcine reproductive and respiratory syndrome virus genotypes I and II (PRRSV-I and PRRSV-II), lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever virus (SHFV) [1]. EAV is the best-characterized arterivirus, although recent studies have increasingly been focused on PRRSV due to its economic importance. Arteriviruses are enveloped, positive-strand RNA viruses that contain a polycistronic genome (12−15 kb) [2−5]. Their replicase proteins are expressed from the open reading frames (ORFs) 1a and 1b that encode two large precursor proteins: the ORF1a-encoded polyprotein pp1a (187−260 kDa) and, following ribosomal frameshifting, the ORF1ab-encoded protein pp1ab (345−422 kDa). Both precursors are processed extensively by three or four ORF1a-encoded endopeptidases into at least 13 mature nonstructural proteins (nsps) [6−16]. The arterivirus proteinases and proteolytic pathways may be compared with those of the related coronaviruses (see Chapters 494 and 546) and roniviruses, which also belong to the order *Nidovirales*, even though the genomes of members of the latter groups are at least twice larger than those of arteriviruses [13,17].

The ***papain-like proteinase 2*** (***PLP2***) in nsp2, originally called ***nsp2 CP*** (for ***cysteine proteinase of nsp2***), is the most C-terminally located member of an array of three (four in SHFV) cysteine proteinase domains that has been identified in the N-terminal 400−800 residues of the arterivirus pp1a/pp1ab (see also Chapters 495 and 496) and it is highly conserved among arteriviruses (Figure 497.1). Its name derives from limited sequence similarity to the active site residues of peptidase clan CA papain-fold enzymes and the relative position of this proteinase in pp1a/pp1ab with respect to upstream PLPs (PLP1α and PLP1β; chapters 495 and 496, respectively). The PLP2 domain was originally identified in nsp2 by bioinformatics and subsequently its activity was verified experimentally for EAV [8], followed by a more recent study on the PRRSV-II PLP2 [18]. The EAV nsp2 N-terminus (pp1a/pp1ab residue Gly261; here and hereafter polyprotein numbering will be used), is liberated by the rapid action of the upstream papain-like cysteine proteinase PLP1β (Chapter 496) [6]. Subsequently, PLP2 mediates the cleavage of the nsp2 C-terminus (the nsp2↓nsp3 site) to generate a 61 kDa cleavage product [7,8]. Similarly, the PRRSV-II PLP2 mediates cleavage of the nsp2↓nsp3 site and, for both proteinases, mutation of either of the putative catalytic Cys and His residues (EAV: Cys270/His332; PRRSV-II (strain VR-2332): Cys437/His506) completely abolished PLP2 activity [8,18].

## Activity and Specificity

The activity of the EAV and PRRSV PLP2s in cleaving the nsp2↓nsp3 site of the replicase polyproteins has been analyzed *in vivo* in infected cells and using eukaryotic expression systems [7,8,18]. The EAV PLP2 cleaves rapidly and probably *in cis*. However, *trans*-cleavage activity was demonstrated in a eukaryotic expression system, albeit with relatively low efficiency [8]. Also for PLP2 of PRRSV-II (strain VR-2332) both *cis*- and *trans*-cleavage activity was demonstrated, and single point mutations of a number of conserved residues in the proteinase domain (*i.e.* Gly438Ala, Asp467Asn, Trp468Gly, Asp471Asn, or Asp471Glu) reduced *trans*- but not *cis*-cleavage

FIGURE 497.1 Multiple alignment of the PLP2 domains of arteriviruses. Shown is a most sequence conserved portion (core) of the PLP2 domain of five arteriviruses: porcine reproductive and respiratory syndrome virus type I and type II (PRRSV1 and PRRSV2, respectively), lactate dehydrogenase-elevating virus (LDV), simian hemorrhagic fever virus (SHFV) and equine arteritis virus (EAV). The alignment was produced with Muscle [37] using the Viralis platform [38] and was prepared for publication with Jalview 2.6.1 [39]. The catalytic Cys and His residues of the PLP2 domain are marked with red #, and conserved Cys residues implicated in Zn-binding with blue *. Conserved and identical residues in all viruses are colored according to Clustal scheme [40] using Jalview 2.6.1. In all arteriviruses the PLP2 protease domain extends downstream of the core and may include functionally important residues. In nsp2 of all arteriviruses except LDV the core domain is flanked by one (EAV) or more residues from the N-terminus. GenBank and/or RefSeq accession numbers of respective virus genome sequences are presented next to the virus acronyms.

efficiency to different extents [18]. Additionally, both EAV and PRRSV PLP2 can remove ubiquitin (Ub) as well as interferon-stimulated gene 15 (ISG15) moieties from different substrates when expressed in mammalian cells [19]. *In vitro* assays showed that PRRSV PLP2 purified from cell lysates can cleave K48-linked polyubiquitin chains [20], while EAV nsp2 PLP2 purified from *E. coli* can cleave both K48- and K63-linked polyubiquitin chains, as well as a linear ubiquitin substrate *in vitro* [21].

The substrate specificity of PLP2 is yet to be defined unequivocally. Originally, the cleavage site was tentatively mapped to the bond between two Gly residues conserved in pp1a/pp1ab of arteriviruses [8,13]. Subsequently, when the deubiquitinating (DUB) activity of PLP2 was demonstrated [19–21] it became more likely that the enzyme's substrate specificity fits the conserved profile of DUBs, implying that PLP2 would cleave downstream rather than upstream of the second Gly [22]. In EAV, cleavage would thus occur at the Gly831-Gly832↓Trp833 sequence, and for PRRSV strain VR-2332 at the Gly1578-Gly1579↓Gly1580 triple Gly motif [23]. Mutagenesis of the putative P2 residue in EAV (Gly831Pro), and the P1 and P2 residues in PRRSV (Gly1578 or Gly1579 to Ser) abolished processing of the nsp2↓nsp3 site [9,18], in accordance with the requirement for Gly residues at the P1 and P2 position of the cleavage site that is typical of DUBs. In addition, several C-terminally truncated versions of nsp2 were detected in MARC-145 cells infected with PRRSV [24], but it remains unclear whether these truncations result from PLP2 autoproteolytic activity or cleavage by a host enzyme. In one particular Vero cell sub-line, EAV nsp2 was also described to be cleaved internally downstream of the PLP2 domain, probably by a host-encoded proteinase [25].

Both large and small deletions in the hydrophilic portion of the region of nsp2, which separates the PLP2 domain and its cleavage site, were found to interfere with cleavage of the EAV nsp2↓nsp3 site [8]. In PRRSV nsp2 the corresponding area is much larger and, in contrast with EAV, large portions of it could be removed or replaced with reporter protein-tags such as GFP or c-myc without affecting the efficiency of polyprotein cleavage or virus viability [18,26]. Replacement of members of a cluster of conserved Cys residues in the C-terminal domain of EAV nsp2 (Cys650, Cys653, Cys657, Cys683, and Cys703) negatively influenced processing of the nsp2↓nsp3 site (Wannee, Snijder & Kikkert, unpublished), and thereby blocked the so-called 'major pathway' for cleavage of EAV replicase polyproteins (see also *Biological Aspects* below) [12]. The association of nsp2 with membranes and its documented interaction with nsp3 (see below) may also modulate the proteolytic activity of PLP2.

## Structural Chemistry

The protease's estimated size, based on comparative sequence analyses (Figure 500.1) and site-directed mutagenesis, is around 135 residues. PLP2 of LDV and EAV was mapped to the very N-terminal domain of nsp2 [8]. On the other hand, comparative sequence analysis predicts that in SHFV, PRRSV-I and PRRSV-II the conserved PLP2 domain is separated from the N-terminus by a hinge region of variable (virus-specific) size, which was verified for PRRSV-II strain VR-2332 [18]. The size of nsp2 ranges from 572 residues in EAV to 1196 residues in PRRSV and it also includes other conserved and variable domains that remain to be characterized. PLP2 is highly conserved among arteriviruses but has only local similarity with viral papain-like proteases [8]. It is instructive to compare it with the PLPα and PLP1β proteinases that are encoded in the nsp1 region of arteriviruses (see Chapters 495 and 496). Unlike the latter two enzymes, PLP2 cleaves quite far downstream (∼500 residues in EAV, ∼1100 residues in PRRSV) of its C-terminal border (Fig. 500.1) and furthermore the catalytic Cys is flanked by Gly, and not by a bulky, hydrophobic residue, which is also found at this position in family C1 peptidases of clan CA (see Chapters 404 and 419). The replacement of Gly271 by Trp completely abolished EAV PLP2 activity towards the nsp2↓nsp3 site [8]. Among the conserved residues in arterivirus PLP2 domains are a number of acidic and Cys residues. Although the replacement of the acidic residues in EAV nsp2 (Asp291, Asp295, Asp296 and Glu297) did not influence PLP2 activity, the substitution of the conserved Cys residues (Figure 500.1), which may form a zinc-binding site, abolished (Cys319, Cys349 and Cys354) or reduced (Cys356) processing of the nsp2↓nsp3 site [8].

## Preparation

The PLP2 domain from EAV nsp2 (residues 261–427) can be purified for *in vitro* studies by cloning it downstream of a maltose binding protein (MBP)-tag in a pMalT vector. After transformation of *E. coli* (BL21 strain) with this plasmid, expression is induced using 1 mM IPTG and after cell lysis protein was bound to amylose resin. Elution is performed with 10 mM maltose, and protein samples are stored at −80°C at ∼1 mg/ml protein concentrations. This protein was soluble and active in DUB assays; however, removal of the MBP-tag caused immediate aggregation and loss of activity (Kikkert & Snijder, unpublished data).

## Biological Aspects

The PLP2 is an arterivirus accessory proteinase that assists the nsp4 main proteinase (see Chapter 691) in the

proteolytic maturation of the replicase polyproteins and the formation of the replication/transcription complex [13]. Cleavage of the nsp2↓nsp3 junction modulates processing of the downstream part of pp1a and pp1ab, which is carried out by the arterivirus serine proteinase (see Chapter 691) [9,12]. Only in the presence of nsp2 in pp1a when this proteinase cleaves the nsp2↓nsp3 junction, the nsp4 proteinase can cleave the nsp4↓nsp5 site at its C-terminus, which initiates the so-called 'major processing pathway'. In truncated pp1a lacking nsp2 the nsp4↓nsp5 site is not cleaved, but the nsp5↓nsp6 and nsp6↓nsp7 sites are cleaved instead ('minor processing pathway') [12].

Arterivirus nsp2 and the downstream nsp3 are membrane-associated proteins [27] that are involved in the modification of endoplasmic reticulum into a network of membrane structures that are presumed to support viral RNA synthesis. Interaction between nsp2 and nsp3 appear to be required for the formation of the typical double-membrane vesicles that are part of this network, although, at least in BHK-21 cells, cleavage of the nsp2↓nsp3 site by PLP2 is not required for this function [25,28,29].

After its release, through its PLP2 domain, nsp2 may also engage in non-replicative functions. Arterivirus PLP2 domains were shown to suppress several antiviral innate immunity signaling pathways [19–21,30], by virtue of their deubiquitinating and de-ISG15ylating activities. Deubiquitination of signal-transducing factors like IκBα [20], RIG-I [21], or other innate immune factors that are subject to ubiquitination likely interrupts signal transduction towards an antiviral state during the early stages of infection. Together with the two upstream proteinases in the arterivirus replicase polyprotein, PLP1α and PLP1β (see Chapters 495 and 496), PLP2 seems to be an important immuno-modulatory protein [31]. A similar function was proposed for coronavirus PLPro domains, which are also deubiquitinating enzymes although they belong to a different ubiquitin-specific proteinase class (see Chapter 494).

## Distinguishing Features

The arterivirus PLP2 is a small cysteine proteinase with a conserved substrate specificity that is produced as part of a larger multi-domain protein. It might have a highly deviant form of the papain-like fold (clan CA), and was suggested to belong to the OTU domain-containing deubiquitinating proteinase superfamily. Its activity is essential for proper proteolytic processing of the arterivirus replicase polyproteins and, additionally, it is associated with the evasion of innate immune responses, by modification of regulatory ubiquitination steps in the signal transduction pathways of this response.

Rabbit antisera against EAV nsp2 have been raised using an N-terminal peptide, a combination of two C-terminal peptides, and a protein fragment encompassing the PLP2 domain purified from *E. coli*. These are available from the authors for research purposes on request.

## Related Peptidases

In evolutionary terms PLP2 is far separated from other papain-like peptidases. Because of their immediate proximity in pp1a/pp1ab of arteriviruses, it is tempting to speculate that PLP1α, PLP1β and PLP2 are the closest parologs. A local statistically non-significant sequence similarity with PLP2s was used to assign the proteolytic activity to a protein family named after the ovarian tumor (OTU) gene product of *Drosophila Melanogaster* [32]. Subsequently, the OTU family was found to include DUBs [33,34], which in turn led to the discovery of the DUB activity in PLP2. Because of these developments, it is now common to treat arterivirus PLP2, as well as another papain-like proteinase of the negative-stranded RNA virus genus *Nairovirus* of the family *Bunyaviridae,* as viral OTU-like proteins [19,35,36] (see also Chapter 499). Still, the evolutionary basis for this link remains uncertain and limited sequence conservation between PLP2s and another DUB family, Ub-C-terminal hydrolases, was also noted [13].

## Further Reading

The reader is referred to the papers of Snijder *et al*. [8], Wassenaar *et al*. [12], Ziebuhr *et al*. [13], Frias-Staheli *et al*. [19], Han *et al*. [18], and Sun *et al*. [20].

## References

[1] Faaberg, K.S., Balasuriya, U.B., Brinton, M.A., Gorbalenya, A.E., Leung, F.C., Nauwynck, H.J., Snijder, E.J., Stadejek, T., Yang, H., Yoo, D. (2012). Family *Arteriviridae*, in: Virus Taxonomy, *Ninth Report of the International Committee on Taxonomy of Viruses*, King, A., Adams, M., Carstens, E., Lefkowitz, E.J., eds., Academic Press, pp. 796–805.

[2] den Boon, J.A., Snijder, E.J., Chirnside, E.D., de Vries, A.A.F., Horzinek, M.C., Spaan, W.J.M. (1991). Equine arteritis virus is not a togavirus but belongs to the coronaviruslike superfamily. *J. Virol.* 65, 2910–2920.

[3] Godeny, E.K., Chen, L., Kumar, S.N., Methven, S.L., Koonin, E.V., Brinton, M.A. (1993). Complete genomic sequence and phylogenetic analysis of the lactate dehydrogenase-elevating virus (LDV). *Virology* 194, 585–596.

[4] Meulenberg, J.J.M., Hulst, M.M., de Meijer, E.J., Moonen, P.L.J.M., den Besten, A., de Kluyver, E.P., Wensvoort, G., Moormann, R.J.M. (1993). Lelystad virus, the causative agent of porcine epidemic abortion and respiratory syndrome (PEARS), is related to LDV and EAV. *Virology* 192, 62–72.

[5] Snijder, E.J., Meulenberg, J.J.M. (1998). The molecular biology of arteriviruses. *J. Gen. Virol.* 79, 961–979.

[6] Snijder, E.J., Wassenaar, A.L.M., Spaan, W.J.M. (1992). The 5′ end of the equine arteritis virus replicase gene encodes a papain-like cysteine protease. *J. Virol.* 66, 7040–7048.

[7] Snijder, E.J., Wassenaar, A.L.M., Spaan, W.J.M. (1994). Proteolytic processing of the replicase ORF1a protein of equine arteritis virus. *J. Virol.* 68, 5755–5764.

[8] Snijder, E.J., Wassenaar, A.L.M., Spaan, W.J.M., Gorbalenya, A.E. (1995). The arterivirus nsp2 protease. An unusual cysteine protease with primary structure similarities to both papain-like and chymotrypsin-like proteases. *J. Biol. Chem.* 270, 16671–16676.

[9] Snijder, E.J., Wassenaar, A.L.M., van Dinten, L.C., Spaan, W.J.M., Gorbalenya, A.E. (1996). The arterivirus nsp4 protease is the prototype of a novel group of chymotrypsin-like enzymes, the 3C-like serine proteases. *J. Biol. Chem.* 271, 4864–4871.

[10] van Dinten, L.C., Wassenaar, A.L.M., Gorbalenya, A.E., Spaan, W.J.M., Snijder, E.J. (1996). Processing of the equine arteritis virus replicase ORF1b protein: identification of cleavage products containing the putative viral polymerase and helicase domains. *J. Virol.* 70, 6625–6633.

[11] van Dinten, L.C., Rensen, S., Spaan, W.J.M., Gorbalenya, A.E., Snijder, E.J. (1999). Proteolytic processing of the open reading frame 1b-encoded part of arterivirus replicase is mediated by nsp4 serine protease and is essential for virus replication. *J. Virol.* 73, 2027–2037.

[12] Wassenaar, A.L.M., Spaan, W.J.M., Gorbalenya, A.E., Snijder, E.J. (1997). Alternative proteolytic processing of the arterivirus replicase ORF1a polyprotein: evidence that NSP2 acts as a cofactor for the NSP4 serine protease. *J. Virol.* 71, 9313–9322.

[13] Ziebuhr, J., Snijder, E.J., Gorbalenya, A.E. (2000). Virus-encoded proteinases and proteolytic processing in the *Nidovirales*. *J. Gen. Virol.* 81, 853–879.

[14] Barrette-Ng, I.H., Ng, K.K.S., Mark, B.L., van Aken, D., Cherney, M.M., Garen, C., Kolodenko, Y., Gorbalenya, A.E., Snijder, E.J., James, M.N.G. (2002). Structure of arterivirus nsp4 – The smallest chymotrypsin-like proteinase with an alpha/beta C-terminal extension and alternate conformations of the oxyanion hole. *J. Biol. Chem.* 277, 39960–39966.

[15] van Aken, D., Zevenhoven-Dobbe, J.C., Gorbalenya, A.E., Snijder, E.J. (2006). Proteolytic maturation of replicase polyprotein pp1a by the nsp4 main proteinase is essential for equine arteritis virus replication and includes internal cleavage of nsp7. *J. Gen. Virol.* 87, 3473–3482.

[16] Fang, Y., Snijder, E.J. (2010). The PRRSV replicase: Exploring the multifunctionality of an intriguing set of nonstructural proteins. *Virus Res.* 154, 61–76.

[17] Gorbalenya, A.E., Enjuanes, L., Ziebuhr, J., Snijder, E.J. (2006). Nidovirales: Evolving the largest RNA virus genome. *Virus Res.* 117, 17–37.

[18] Han, J., Rutherford, M.S., Faaberg, K.S. (2009). The porcine reproductive and respiratory syndrome virus nsp2 cysteine protease domain possesses both trans- and cis-cleavage activities. *J. Virol.* 83, 9449–9463.

[19] Frias-Staheli, N., Giannakopoulos, N.V., Kikkert, M., Taylor, S.L., Bridgen, A., Paragas, J., Richt, J.A., Rowland, R.R., Schmaljohn, C.S., Lenschow, D.J., Snijder, E.J., García-Sastre, A., Virgin, H.W. (2007). Ovarian tumor domain-containing viral proteases evade ubiquitin- and ISG15-dependent innate immune responses. *Cell Host Microbe* 2, 404–416.

[20] Sun, Z., Chen, Z., Lawson, S.R., Fang, Y. (2010). The cysteine protease domain of porcine reproductive and respiratory syndrome virus nonstructural protein 2 possesses deubiquitinating and interferon antagonism functions. *J. Virol.* 84, 7832–7846.

[21] van Kasteren, P.B., Beugeling, C., Ninaber, D.K., Frias-Staheli, N., van Boheemen, S., Garcia-Sastre, A., Snijder, E.J., Kikkert, M. (2012). Arterivirus and nairovirus ovarian tumor domain-containing deubiquitinases target activated RIG-I to control innate immune signaling. *J. Virol.* 86, 773–785.

[22] Komander, D., Clague, M.J., Urbé, S. (2009). Breaking the chains: structure and function of the deubiquitinases. *Nat. Rev. Mol. Cell Biol.* 10, 550–563.

[23] Han, W., Wu, J.J., Deng, X.Y., Cao, Z., Yu, X.L., Wang, C.B., Zhao, T.Z., Chen, N.H., Hu, H.H., Bin, W., Hou, L.L., Wang, L.L., Tian, K.G., Zhang, Z.Q. (2009). Molecular mutations associated with the *in vitro* passage of virulent porcine reproductive and respiratory syndrome virus. *Virus Genes* 38, 276–284.

[24] Han, J., Rutherford, M.S., Faaberg, K.S. (2010). Proteolytic products of the porcine reproductive and respiratory syndrome virus nsp2 replicase protein. *J. Virol.* 84, 10102–10112.

[25] Snijder, E.J., van Tol, H., Roos, N., Pedersen, K.W. (2001). Non-structural proteins 2 and 3 interact to modify host cell membranes during the formation of the arterivirus replication complex. *J. Gen. Virol.* 82, 985–994.

[26] Han, J., Liu, G., Wang, Y., Faaberg, K.S. (2007). Identification of nonessential regions of the nsp2 replicase protein of porcine reproductive and respiratory syndrome virus strain VR-2332 for replication in cell culture. *J. Virol.* 81, 9878–9890.

[27] van der Meer, Y., van Tol, H., Krijnse Locker, J., Snijder, E.J. (1998). ORF1a-encoded replicase subunits are involved in the membrane association of the arterivirus replication complex. *J. Virol.* 72, 6689–6698.

[28] Pedersen, K.W., van der Meer, Y., Roos, N., Snijder, E.J. (1999). Open reading frame 1a-encoded subunits of the arterivirus replicase induce endoplasmic reticulum-derived double-membrane vesicles which carry the viral replication complex. *J. Virol.* 73, 2016–2026.

[29] Posthuma, C.C., Pedersen, K.W., Lu, Z., Joosten, R.G., Roos, N., Zevenhoven-Dobbe, J.C. (2008). Formation of the arterivirus replication/transcription complex: a key role for nonstructural protein 3 in the remodeling of intracellular membranes. *J. Virol.* 82, 4480–4491.

[30] Li, H., Zheng, Z., Zhou, P., Zhang, B., Shi, Z., Hu, Q., Wang, H. (2010). The cysteine protease domain of porcine reproductive and respiratory syndrome virus non-structural protein 2 antagonizes interferon regulatory factor 3 activation. *J. Gen. Virol.* 91, 2947–2958.

[31] Yoo, D., Song, C., Sun, Y., Du, Y., Kim, O., Liu, H.C. (2010). Modulation of host cell responses and evasion strategies for porcine reproductive and respiratory syndrome virus. *Virus Res.* 154, 48–60.

[32] Makarova, K.S., Aravind, L., Koonin, E.V. (2000). A novel superfamily of predicted cysteine proteases from eukaryotes, viruses and *Chlamydia pneumoniae*. *Trends Biochem. Sci.* 25, 50–52.

[33] Balakirev, M.Y., Tcherniuk, S.O., Jaquinod, M., Chroboczek, J. (2003). Otubains: a new family of cysteine proteases in the ubiquitin pathway. *EMBO Reports* 4(5), 517−522.

[34] Evans, P.C., Smith, T.S., Lai, M.J., Williams, M.G., Burke, D.F., Heyninck, K., Kreike, M.M., Beyaert, R., Blundell, T.L., Kilshaw, P.J. (2003). A novel type of deubiquitinating enzyme. *J. Biol. Chem.* 278(25), 23180−23186.

[35] Capodagli, G.C., McKercher, M.A., Baker, E.A., Masters, E.M., Brunzelle, J.S., Pegan, S.D. (2011). Structural analysis of a viral ovarian tumor domain protease from the Crimean-Congo hemorrhagic fever virus in complex with covalently bonded ubiquitin. *J. Virol.* 85, 3621−3630.

[36] James, T.W., Frias-Staheli, N., Bacik, J.P., Levingston Macleod, J.M., Khajehpour, M., Garcia-Sastre, A., Mark, B.L. (2011). Structural basis for the removal of ubiquitin and interferon-stimulated gene 15 by a viral ovarian tumor domain-containing protease. *Proc. Natl. Acad. Sci. USA* 108, 2222−2227.

[37] Edgar, R.C. (2004). MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5, 113.

[38] Gorbalenya, A.E., Lieutaud, P., Harris, M.R., Coutard, B., Canard, B., Kleywegt, G.J., Kravchenko, A.A., Samborskiy, D.V., Sidorov, I.A., Leontovich, A.M., Jones, T.A. (2010). Practical application of bioinformatics by the multidisciplinary VIZIER consortium. *Antiviral Res.* 87(2), 95−110.

[39] Waterhouse, A.M., Procter, J.B., Martin, D.M., Clamp, M., Barton, G.J. (2009). Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics* 25(9), 1189−1191.

[40] Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G. (2007). Clustal W and Clustal X version 2.0. *BMC. Bioinformatics* 23, 2947−2948.

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