Structure, Function, and Evolution of the Crimean-Congo Hemorrhagic Fever Virus Nucleocapsid Protein


Crimean-Congo hemorrhagic fever virus (CCHFV) is an emerging tick-borne virus of the Bunyaviridae family that is responsible for a fatal human disease for which preventative or therapeutic measures do not exist. We solved the crystal structure of the CCHFV strain Baghdad-12 nucleocapsid protein (N), a potential therapeutic target, at a resolution of 2.1 Å. N comprises a large globular domain composed of both N- and C-terminal sequences, likely involved in RNA binding, and a protruding arm domain with a conserved DEVD caspase-3 cleavage site at its apex. Alignment of our structure with that of the recently reported N protein from strain YL04057 shows a close correspondence of all folds but significant transposition of the arm through a rotation of 180 degrees and a translation of 40 Å. These observations suggest a structural flexibility that may provide the basis for switching between alternative N protein conformations during important functions such as RNA binding and oligomerization. Our structure reveals surfaces likely involved in RNA binding and oligomerization, and functionally critical residues within these domains were identified using a minigenome system able to recapitulate CCHFV-specific RNA synthesis in cells. Caspase-3 cleaves the polypeptide chain at the exposed DEVD motif; however, the cleaved N protein remains an intact unit, likely due to the intimate association of N- and C-terminal fragments in the globular domain. Structural alignment with existing N proteins reveals that the closest CCHFV relative is not another bunyavirus but the arenavirus Lassa virus instead, suggesting that current segmented negative-strand RNA virus taxonomy may need revision.

CCHFV is a member of the Bunyaviridae family, and together with members of the Arenaviridae and Orthomyxoviridae families, these viruses are known as segmented negative-strand RNA viruses (sNSVs) by virtue of their multiple genome segments. The Bunyaviridae family contains over 350 named isolates classified within five genera, namely, Hantavirus, Nairovirus, Orthobunyavirus, Phlebovirus, and Tospovirus (41). CCHFV is a nairovirus, with a genome comprising three RNA segments: the small (S), medium (M), and large (L) segments. The S segment encodes the nucleocapsid protein (N), the M segment encodes the viral glycoproteins, and the L segment encodes an RNA-dependent RNA polymerase (RdRp); the L protein.

The genomes of sNSVs do not exist as naked RNAs but instead are encapsidated by the viral N protein to form ribonucleoprotein (RNP) complexes. RNPs associate with their cognate RdRp to form active templates for viral RNA synthesis, resulting in generation of encapsidated replication products and unencapsidated mRNAs. Genome encapsidation is also required for RNP packaging into progeny virus particles, and for bunyaviruses, virus assembly is mediated through direct association between the RNP and viral glycoproteins (18, 31, 39, 42, 44). RNP formation is thus essential for virus multiplication and therefore represents a potential therapeutic target.

In addition to RNP formation and virus assembly, the N proteins of bunyaviruses are implicated in other important functions, many of which relate to interactions with components of the host cell, including the cytoskeleton (3, 35–37, 43), cellular RNAs (26, 28), the translation machinery (8, 27), and mediators of the innate immune response (20, 30). Specifically for CCHFV, the N protein interacts with the cellular antiviral defense factor MxA (2) and recently was shown to act as a substrate for the apoptosis mediator caspase-3 (19), although the relevance of caspase-3 cleavage to the virus life cycle is unknown.

We present the 2.1-Å crystal structure of the N protein from CCHFV strain Baghdad-12. The CCHFV N structure we present displays significant differences in domain position compared to the recently reported structure of N from CCHFV strain YL04057.

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(15), isolated from China, and these differences may have important functional consequences, as discussed below. The structure reveals interfaces possibly involved in the critical N protein activities of N-N oligomerization and RNA binding, and these functions were tested in a minigenome assay in mammalian cells. In addition, the N structure reveals strong and unexpected structural homologies with the N protein of the arenavirus Lassa virus (LASV), suggesting previously unappreciated aspects of sNSV phylogenetics, with the conclusion that current sNSV taxonomy may need revision.

MATERIALS AND METHODS

Structure determination. The CCHFV N protein from the Baghdad-12 strain was expressed, purified, and crystallized as previously described (6). The N protein structure was solved by single isomorphous replacement with anomalous scattering (SIRAS) using a mercury derivative obtained by soaking natively grown CCHFV N crystals, suspended under the original crystallization conditions, with 5 mM thimerosal for 20 min (6). Eight prominent mercury peak sites were found, and phases from these were assigned to the native set by use of autoSHARP (50). Density modification was implemented by PARROT (9), after which the automated model building program Buccaneer (1) was used to build 85% of the model. The model was then subjected to iterative cycles of manual building in COOT (12), and refinement in REFMAC (29) with TLS parameters applied until 98% of the protein molecules had been modeled. The TLS parameters were determined by the TLSMD server (33), which defined the globular and arm domains as separate rigid bodies. The ARP/wARP solvent program (21) was used to initially locate the bulk of water molecules, followed by manual building of the remaining solvent molecules in COOT. The fully refined model has an Rwork value of 18.4% and an Rfree value of 23.4% (Table 1) and was validated using MolProbity (7).

Plasmid construction. The CCHFV N protein cDNA sequence in the previously described plasmid pC-N (4) was mutated using a site-directed mutagenesis approach based on structural and phylogenetic data. Selected residues were mutated to alanine (A), with the following exceptions: aspartic acid residue 272 (in the conserved DEVD caspase cleavage site) was mutated to either a glycine (G) or a glutamate (E), while isoleucine residue 304 was mutated to a glycine. The CCHFV N coding sequence was amplified from pC-N by using forward and reverse primers that introduced Clal and Xhol sites at the 3' and 5' ends of the sequence, respectively. The PCR product was ligated into pCR-Blunt II-TOPO (Invitrogen), and the coding sequence was mutated using Pfui Turbo polymerase as recommended by the manufacturer (Agilent Technologies). Where two mutations were introduced into the coding sequence, the mutagenesis was performed using a tandem approach. The mutated coding sequence was restricted out of the cloning vector by use of Clal/Xhol and was ligated into a Clal/Xhol-digested pC-N backbone. All clones were verified by sequencing.

Replicon assay. Plasmid sequences and detailed cloning strategies are available upon request. Briefly, the CCHFV strain Ibar200 M segment minigenome (T7-M-Rep) was generated by replacing the M open reading frame (ORF) with the RepHila luciferase gene (Promega), and the corresponding sequence was cloned into a vector designed to express viral sense RNAs from a T7 RNA polymerase promoter, as previously described (4). The V5-tagged L protein codons (4) were optimized for expression in human cells (Genearnt), and the corresponding sequence was cloned into pcAGGS (PC-V5-L). BSR/T7 cells (Klaus Conzelmann, Max von Pettenkofer Institut, Munich, Germany) were seeded in 48-well plates 1 day prior to transfection, at a density of 3.5 × 10^4 cells per cm^2. The cells in each well were transfected with 125 ng of PC-V5-L, 250 ng of wild-type PC-N or one of its mutants, 15 ng of T7-M-RepHila, and 15 ng of a firefly luciferase internal control plasmid (pGL3; Promega). Renilla and firefly luciferase activities were measured with a Dual-Glo luciferase assay system (Promega) on a Synergy 4 microplate reader (Biotek). CCHFV M minigenome Renilla luciferase activities were normalized over the control firefly luciferase activity and are reported as percentages of wild-type N activity.

In vitro caspase-3 cleavage and analysis. Caspase-3 was expressed and purified according to previously published methods (10), using a plasmid encoding the full-length caspase-3 protein (53) fused to a C-terminal 6×His tag (Addgene). Expression and purification of caspase-3 were confirmed by SDS-PAGE, and its concentration was estimated by the Bradford assay (protein dye reagent; Bio-Rad) using bovine serum albumin (BSA) standards, after which the purified protein was concentrated to 10 mg ml^-1. To measure caspase-3 cleavage of CCHFV N proteins with both wild-type (DEVd) and mutant (DEVe and DEVg) caspase-3 cleavage sites, a range of in vitro reactions were performed. Different molar ratios of caspase-3 protease and CCHFV N protein were added to 1.5-ml microcentrifuge tubes containing caspase-3 digestion buffer (20 mM piperazine-N,N'-bis(2-hydroxypropanesulfonic acid) (PIPES), pH 7.2, 200 mM NaCl, 20% sucrose, 0.2% (wt/vol) 3-[(3-cholamidopropyl)-dimeth-
the core of the globular domain, which in turn are surrounded by helices from the N-terminal half. The monomers are packed in a head-to-tail arrangement, with the interface between monomers comprising helices α6 and α7 and helices α21 and α22 within the globular domain.

Analysis of the electrostatic surface potential of the N protein (Fig. 1D) revealed a continuous positively charged region located under the arm domain, and we refer to this region as the “platform.” An additional isolated area of positive charge is adjacent to a deep basic crevice, and this region is referred to as the “pocket.” Residues that comprise the putative RNA binding platform and pocket are conserved in all CCHFV isolates.

Structural comparison of CCHFV N proteins from strains Baghdad-12 and YL04057. The globular domains from strains YL04057 and Baghdad-12 are very similar, with a Cα root mean square deviation (RMSD) of 0.975 Å for 357 residues with 92.16% sequence identity. In contrast, and most significantly, while the fold of the arm domain is the same (Cα RMSD of 0.642 Å, 99 residues, 77.86% identity), the position of the arm domain is radically altered; with respect to the globular domain, the arm is rotated by about 180 degrees, and the apex of the loop is shifted by a distance of 39.59 Å (at the Cα position of Asp266, at the apex of the arm, in each protein) (Fig. 1B). The main secondary structural difference that may account for this transposition is the division of a long helix in strain YL04057 into two shorter helices (α17 and α18) in strain Baghdad-12 (Fig. 1A), which may imply flexibility at the base of the arm.

Interestingly, comparison of the primary sequences of strains Baghdad-12 and YL04057 reveals 29 amino acid differences among 481 positions (Fig. 1C). Only nine of these are nonconservative, although the amino acid sequences at the division of helices 17 and 18 are identical, which could argue against strain differences being the major determining factor in arm position.

Similarity to other segmented RNA virus N proteins. To provide insight into bunyavirus phylogenetics, we compared our CCHFV N structure to the recently reported structure of N from RVFV (14, 38), a phlebovirus. Alignment using the DALI server revealed essentially no structural homology between the CCHFV and RVFV N globular domains (Z score = 1.7), and zero similarity was detected with the arm domain. Interestingly, the DALI server identified strongest structural similarity with the N protein of the Arenaviridae family member LASF (5, 16, 17, 34), specifically within N-terminal residues 1 to 340, which comprise the RNA binding domain of LASF N (Fig. 2A) (17). Structural homology was identified for all forms (RNA-free and RNA bound) of LASF N deposited in the PDB (16, 17, 34), with Z scores falling between 12.4 and 15.3. The globular domains from the CCHFV and RNA-free LASF N proteins superposed with a Cα root mean square deviation of 3.38 Å for 237 residues with 80.1% sequence identity.

FIG 1 Structure of the CCHFV N protein. (A) Structure of CCHFV N monomer in ribbon representation. Helices are shown in gray for the N-terminal portion and in dark blue for the C-terminal contribution to the globular domain and are shown in purple for the arm. The red sphere marks the N terminus, and the gold sphere marks the C terminus. Colored arrowheads show the caspase-3 DEVD cleavage motif (yellow) and the division of helices 17 and 18 (green). Panels A, B, and D were generated using PyMol. (B) Superposition (center) of the Baghdad-12 N structure (blue) and the YL04057 N structure (red) reveals very similar globular domains, whereas the arm has the same fold but is shifted relative to the globular domain. An expanded view of the region between the arm and the globular domain for each strain is shown, with relevant helices labeled. Numbering of helices for Baghdad-12 is as shown in panel C, whereas numbering of helices for YL04057 is according to the work of Guo et al. (15). (C) Primary amino acid sequences of N proteins of both strains of CCHFV, with the secondary structure shown schematically. The figure shown was generated with ALINE (4a). The DEVD motif is highlighted in yellow, strain differences are highlighted in red, residues missing in the electron density are highlighted in black, and the kink between helices 17 and 18 (at Ser294) is highlighted in green. (D) The electrostatic surface potential (generated with APBS) suggests a positively charged RNA binding platform adjacent to the arm (47a), and rotation by 180 degrees suggests a positively charged cleft that we term the “pocket.” The scale bar shows the contour levels for the electrostatic potential at the solvent-exposed surface, in kT/e.
Surprisingly, this suggests that CCHFV is more closely related to the arenavirus LASV than to the bunyavirus RVFV. This proposal is supported by a phylogenetic analysis of sNSV L and N sequences (Fig. 2B and C), which similarly implies that nairoviruses are more closely related to arenaviruses than to any other bunyavirus genera.

Potential for a gated RNA binding mechanism. The electrostatic charge distribution on the CCHFV N surface revealed two possible RNA binding regions: the platform and the pocket. Structural comparison of N proteins from strains Baghdad-12 and YL04057 suggested that the arm is free to explore a range of conformations, which could significantly alter surface electrostatics and therefore RNA binding. In addition, the flexible loop between the arm and the globular domain (residues 183 to 191; not visible in either the YL04057 or Baghdad-12 N structure) could become ordered on RNA binding. Thus, the arm domain may “gate” RNA binding, similar to the LASV and RVFV gate (see Discussion).

The N-N dimer interface. The formation of viral RNPs presumably relies on N-N interactions, and we therefore examined potential interacting surfaces that could mediate this function.
The CCHFV N protein was expressed and purified as an RNA-free monomer (6). However, as described above, the AU in the C2 space group comprises two monomers (Fig. 3B), and it is plausible that the interface seen between these two non-crystallographic symmetry (NCS)-related molecules represents a biologically relevant dimer interface.

Analysis of the potential dimer interface by use of the PISA server reveals an average buried surface area of 1,015 Å², a $\Delta G$ value of −9.5 kcal/mol, and a complexation significance score (CSS) of 0.0 (a score of 1 suggests a stable interface, and a score of 0 suggests a nonstable interface, i.e., crystal packing). For comparison, the oligomeric interfaces present in the RVFV N structure have an average buried surface area of 1,643 Å², a $\Delta G$ value of −18.5 kcal/mol, and a CSS of 1.0. However, lattice contacts within the same RVFV crystal form present an average buried surface area of 437 Å² and a $\Delta G$ value of −3.9 kcal/mol; thus, the CCHFV N interface was scored at values intermediate between the two. PISA analysis thus suggests that even the largest interface is not

**FIG 3 In vivo effects of site-directed CCHFV N protein mutants. (A)** Residues selected for mutation and testing in the minireplicon system are highlighted on the structure. Residues in the RNA binding platform are highlighted in green, residues that comprise the RNA binding pocket are shown in blue, the possible dimer interface is shown in red, and D269 of the DEVD motif is shown in orange. Alterations of residues K132, Q300, and K411 abrogated minigenome activity, and these residues are shown in magenta. (B) Ribbon representation of two protomers within the crystal lattice, suggesting the putative dimer interface investigated using the minireplicon system. (C) Histogram showing reporter gene expression for mutants, colored as described above, normalized to that of wild-type (WT) N. (D) Western blot analysis to assess relative expression levels of N protein mutants from equivalent quantities of cells expressing the minireplicon system, using a polyclonal antibody raised against the CCHFV N protein.
sufficient to generate a stable interface in solution. However, this interface may represent part of the authentic oligomerization domain, with the possibility that a conformational change on RNA binding may reveal a more extensive or rearranged interface.

Functional analysis of selected N protein residues by use of a CCHFV minigenome system. The structure of the CCHFV N protein revealed the identity of amino acids that could potentially be involved in the critical activities of RNA binding and N-N oligomerization. To investigate the role of N protein residues in these activities, we used a CCHFV minireplicon system in which reporter gene expression was promoted by formation of RNP templates from RNA and N protein in mammalian cells (4). A panel of N proteins bearing mutations within the proposed RNA binding surfaces (Fig. 3A), the putative oligomerization interface (Fig. 3B), or the DEVD motif (Fig. 3A) was generated, with residue selection based on position within the N structure and conservation within available CCHFV N sequences. Reporter expression in the minigenome system (Fig. 3C) depends on the ability of the N protein to both bind RNA and multimerize to form an RNP template; therefore, a loss of reporter expression will reflect deficiencies in either of these activities. Of nine mutants with alterations within the RNA binding platform, six exhibited essentially unchanged replicon activity (K91A, K98A, E112A, R140A, S149A, and Y470A); however, the three remaining mutants showed replicon activity that was either significantly reduced (K90A) or effectively abrogated (K132A and Q300A). Of the selected mutants with mutations of positively charged residues within the RNA binding pocket, the K342A, K343A, and H453A mutants exhibited nearly wild-type activity; however, the K411A and H456A mutants resulted in abrogated and significantly reduced RNP activity, respectively. Additional mutants, i.e., the E387A, I304G, and W313A mutants, were also generated but were expressed poorly or undetectably in transfected cells (Fig. 3D) and were therefore not analyzed in the minigenome system.

Taken together, the results of the analysis identified three separate residues (K132, Q300, and K411) that were individually essential for replicon activity and another two residues (K90 and H456) whose mutation resulted in a significantly reduced N functionality. Because of their charge characteristics and specific location on the N protein surface, we propose that these residues likely play a role in RNA binding. Interestingly, residue K411 was recently described as contributing to DNase activity of the CCHFV N protein (15), whereas our results favor a direct role in CCHFV gene expression.

We also investigated the functional relevance of the crystallographic dimer interface (Fig. 3B) by using both single-residue mutants (E108A and K114A mutants) and double-residue mutants (K354A/E108A, K357A/E112A, K358A/R140A, and E361K/K114A mutants). None of these mutants resulted in abrogation of RNP function, suggesting that none of the altered amino acids were essential for N protein oligomerization. However, the K354A/E108A and K357/E112A double mutants showed a drop in RNP activity, suggesting that the corresponding residues may play roles in RNP assembly, possibly by promoting oligomerization.

The mutants with altered DEVD motifs (DEVG and DEVE), which are not cleavable by caspase-3 (see below), promoted levels of minigenome activity that were indistinguishable from that of wild-type N. While caspase-3 cleavage may have an effect on the life cycle of infectious virus, we do not expect to see this recapitu-
gene expression, we propose are involved in RNA binding. In support of our identification of these putative RNA binding regions, the CCHFV N globular domain shows a high degree of structural similarity with the N-terminal RNA binding domain of the LASV N protein. The consequences of this close structural relationship are discussed further below.

In LASV, there is a proposed gating mechanism of RNA binding where helices α5 and α6 are repositioned to reveal the RNA binding surface (17). The concept of a flexible arm being involved in gating RNA binding has also been proposed for RRVFV N, which has an N-terminal arm interacting with an oligomerization groove on adjacent monomers and consequently exposing an RNA binding cleft (14). CCHFV N may operate via a similar mechanism, although the structural elements involved in the gating process are likely different. The position of helix α5 in LASV corresponds to the beginning of the flexible loop leading to the CCHFV arm domain; thus, one possibility is that the arm of CCHFV N may be involved in switching between RNA-bound and unbound conformations.

Structural alignment (Fig. 2A) revealed that the CCHFV RNA binding pocket corresponds to the domain within LASV N that was initially thought to bind the cap (34) but was later suggested to represent a binding pocket involved in the interaction with a single nucleotide of a bound RNA strand (17). This raises the possibility either that CCHFV N binds RNA via two surfaces (platform and pocket) or that conformational rearrangements create a continuous RNA binding surface. It is quite possible that a combination of arm movement and structural rearrangements is required in order to reveal the appropriate RNA binding surfaces, which may not be evident in the apo crystal structures.

Because we do not know the length of RNA bound by each N monomer, or indeed the oligomeric form of N in the RNP, we cannot currently discriminate between any plausible RNA binding mechanisms or deduce what any required conformational changes might be. However, it seems reasonable, based on previous studies of RNP formation, to anticipate more details of the RNA binding mechanism to be revealed by a structure of the N protein in complex with RNA, possibly via a gating mechanism analogous to that seen for LASV.

The possibility that the arm domain can adopt different positions is supported by comparison of our N structure for strain Baghdad-12 with that for strain YL04057, reported recently (15). While the globular domains align very closely, the arm domains adopt radically different positions, being rotated by nearly 180 degrees and with the arm apex being translated by 40 Å (Fig. 1B). This rearrangement may have important consequences, as the two arm positions may represent interchangeable forms that possess different activities in critical N protein functions such as oligomerization or RNA binding. However, one possibility is that the arm positions are dictated by the different primary sequences of the respective strains.

Strain differences could account for a change in the preferred lowest energy state. However, the arm is more likely to be more mobile and free to adopt a number of conformations, taking into account the likelihood that an exposed α helix is not a stable structure in isolation. The single α helix linking the globular and arm domains is unlikely to provide a rigid link. Alternatively, these differences may have been forced by crystal packing. However, should the strain differences we observed also be reflected in solution, one consequence of the shifted arm position and sequence differences between the two N structures is an altered distribution of electrostatic surface potential, which may affect the RNA binding ability of the respective proteins. It is possible that the arm represents part of a gating mechanism allowing a switch between RNA-bound and unbound states. However, it is also plausible that the switch in arm position is responsible for conversion of monomeric N into higher-order multimers, a property that is required for RNP formation.

In addition to the radically different arm positions posing possible functional significance, the differing arm conformations of these two strains may also have a bearing on strategies for struc-
structure-based design of small-molecule inhibitors for use as antivi-

rals.

A striking feature of the CCHFV N structure is that the arm
domain displays a DEVD caspase cleavage motif at its apex, in the
most accessible position on the entire molecule (Fig. 1A). This
exposed position, along with its strict conservation in all CCHFV
strains, suggests that the virus has evolved to present the cleavage
motif to the cellular environment, which is somehow beneficial to
the virus life cycle. If possession of the exposed cleavage site were
not beneficial to the virus or if caspase cleavage were a host defense
mechanism, a fast-mutating RNA virus such as CCHFV would be
predicted to quickly eliminate the motif. The functional signifi-
cance of this caspase cleavage site is therefore an intriguing feature
of the CCHFV N protein. To investigate the fate of the N protein
fragments following cleavage, we performed caspase cleavage of
the N protein in vitro and showed that the cleavage products re-

mained associated as a single unit. This raises the interesting pos-
sibility that N protein functions may remain unaltered following
cleavage. The cleaved N protein could of course have an altered
tertiary or quaternary structure, which may influence function in
a variety of ways, including interaction with different protein part-
ers, the adoption of different oligomeric states, or alteration of
RNA binding affinity. In order to best understand the functional
relevance of this DEVD motif in the CCHFV life cycle, we need to
manipulate the CCHFV genome with a view to studying the con-
sequence of such a change in the context of a live virus infection of
intact organisms. Unfortunately, such a system currently does not
exist.

Intriguingly, the nucleocapsid protein (NP) of human-infecting
strains of influenza A virus also possesses caspase cleavage sites
which have been shown to possess important roles in the virus life

cycle (51, 52). Infectious influenza viruses bearing mutations that
abrogate caspase cleavage at an N-terminal recognition site could
not be rescued, indicating that such alterations are lethal to virus
viability, whereas mutations within a C-terminal caspase cleavage
site rapidly reverted to the wild type to restore cleavability. These
findings establish an important precedent that links caspase cleav-
ability with virus fitness, and thus pathogenesis, suggesting a sec-
ond functional surface (in addition to the RNA binding site) that
could be targeted by antivirals.

Structural comparisons indicate that the CCHFV N protein
globular domain exhibits a high degree of homology with the N-
terminal domain of the N protein of LASV, a member of the Are-
naviridae family, whose members uniformly possess two RNA
segments. In contrast, CCHFV N shows negligible structural simi-
ilarity with the only other bunyavirus (RVFV) N protein for which
high-resolution structural data are available. The finding that
these bunyavirus N proteins appear essentially structurally unre-
lated yet the CCHFV N protein shows extremely high homology
with the N protein from a virus of a different taxonomic family
with a different number of genome segments is intriguing. Previ-
ous phylogenetic analyses based on L protein sequences have also
described a close relationship between nairoviruses and arenavi-

ruses (48), and this conclusion is further supported by the phylo-
genetic analysis of N and RdRp protein sequences presented here
(Fig. 2B and C). Further supporting this close relationship, arenavi-
ruses and nairoviruses also possess unique aspects of cellular
biology that are absent from other bunyaviruses, such as the de-
pendence of cellular SK1-1 protease processing of their respective
glycoprotein precursors (23, 49). However, the high degree of
structural similarity we observed between the CCHFV and LASV
N proteins is the most compelling evidence yet that arenaviruses
have an ancestor in common with a current or past member of the
Nairovirus genus. This suggests that the current classification of all
three-segment negative-strand RNA viruses as bunyaviruses may
be oversimplified and that the evident diversity within the Bunya-
viridae family may warrant reevaluation of its current taxonomic
status.

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REFERENCES


Crimean–Congo hemorrhagic fever virus nucleocapsid protein to perinuclear
hemorrhagic fever virus nucleocapsid protein to perinuclear


vealed by X-ray crystallography, small-angle X-ray scattering, and elec-
6. Carter SD, Barr JN, Edwards TA. 2012. Expression, purification, and

crystallization of the Crimean–Congo hemorrhagic fever virus nucleocap-
573.
7. Chen VB, et al. 2010. MolProbity: all-atom structure validation for mac-

12–21.
virus nucleocapsid protein (N) and ribosomal protein S19 (RPS19). J.
10. Denault JB, Salvesen GS. 2003. Expression, purification, and character-
Crimean–Congo hemorrhagic fever virus genomics and global diversity. J.
Evaluation of serum levels of interleukin (IL)-6, IL-10, and tumor necrosis
193:941–944.
nucleoprotein suggests a mechanism for its assembly into ribonucleoprotein

16. Hastie KM, Kimberlin CR, Zandonatti MA, MacRae IJ, Saphire EO.