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## Structural Characterization and Validation of Crimean-Congo Haemorrhagic Fever Virus Nucleoprotein Using Bioinformatics Approaches

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**Abstract:** Crimean-Congo haemorrhagic fever virus (CCHFV) is an important viral pathogen, and characterization of its structural proteins contributes to a better understanding of viral molecular features. In this study, the CCHFV nucleoprotein was subjected to sequence analysis, three-dimensional structure modelling, structural validation, and physicochemical characterization. The nucleoprotein sequence consisting of 482 amino acids was retrieved from the NCBI database and used for homology-based structural modelling. The predicted model was generated using the CCHFV nucleoprotein X-ray crystallographic structure (template: 4aqf.2.A), showing 100% query coverage, 97.03% sequence similarity, and a Global Model Quality Estimate (GMQE) score of 0.93. Structural validation demonstrated the reliability of the model, with 97.03% residues present in the Ramachandran favoured region and 0.00% residues identified as outliers. The model quality assessment further showed a MolProbity score of 2.03, clash score of 2.55, and QMEANDisCo global score of  $0.87 \pm 0.05$ , indicating good structural agreement with the template structure. Physicochemical analysis revealed that the nucleoprotein has a molecular weight of 53,891.36 Da, theoretical isoelectric point of 8.47, positive overall charge, instability index of 31.44, aliphatic index of 76.33, and GRAVY value of  $-0.414$ . The obtained structural model and characterization data provide a foundation for further investigations of CCHFV nucleoprotein structure and function.

**Keywords:** Ticks; Tick-borne diseases, MicroRNAs, Small non-coding RNAs, Vector competence, Tick-pathogen interactions, Tick immunity, Pathogen transmission, Host-vector interface, Molecular biomarkers, RNA-based control strategies

### Introduction

Crimean-Congo haemorrhagic fever virus (CCHFV) is an important emerging tick-borne viral pathogen responsible for Crimean-Congo haemorrhagic fever (CCHF), a severe zoonotic disease characterized by fever, vascular dysfunction, and haemorrhagic manifestations. The virus is widely distributed across Africa, Asia, the Middle East, and parts of Europe, where transmission occurs mainly through Hyalomma ticks and contact with infected animals or tissues. Due to its broad geographic distribution, outbreak potential, and limited availability of specific therapeutic options, CCHFV remains a significant public health concern (Hawman & Feldmann, 2023).

CCHFV belongs to the genus Orthonairovirus within the family Nairoviridae and possesses a tripartite negative-sense RNA genome consisting of small (S), medium (M), and large (L) segments. The S segment encodes the nucleoprotein (NP), the M segment encodes the glycoprotein precursor, and the L segment encodes the viral RNA-dependent RNA

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polymerase. Among these viral proteins, the nucleoprotein is highly abundant and performs essential functions in viral replication by binding viral RNA and forming ribonucleoprotein complexes involved in genome organization, protection, and replication (Zivcec et al., 2016).

The CCHFV nucleoprotein exhibits a conserved structural organization that contributes to its biological function. Structural investigations have demonstrated that nucleoprotein contains structural regions involved in RNA interaction and oligomeric organization, providing insights into the mechanisms associated with viral genome packaging and replication. The experimentally determined structure of the nucleoprotein has improved understanding of its molecular architecture and evolutionary conservation among related nairoviruses (Carter et al., 2012). Further studies have shown that CCHFV nucleoprotein possesses dual RNA-binding modes, interacting with different RNA substrates, suggesting structural flexibility that may contribute to viral genome encapsidation and replication processes (Jeeva et al., 2017).

Advances in structural bioinformatics have enabled detailed analysis of protein structures through computational approaches such as homology modelling, structural validation, and physicochemical characterization. Homology modelling allows prediction of three-dimensional protein structures using experimentally resolved templates, while validation approaches, including Ramachandran plot analysis and model quality assessment, provide information regarding structural reliability. These computational methods are valuable for investigating viral proteins and understanding their molecular characteristics.

The CCHFV nucleoprotein is also considered an important molecular component due to its conservation and immunogenic properties. Studies have demonstrated that nucleoprotein contributes to host immune recognition and represents an important component for diagnostic and immunological investigations (Pirinç & Doymaz, 2024). Therefore, detailed characterization of its structural and physicochemical properties is essential for improving understanding of CCHFV molecular biology.

In the present study, the CCHFV nucleoprotein was analysed using bioinformatics-based approaches involving sequence analysis, three-dimensional homology modelling, structural validation, and physicochemical characterization. The predicted structural model was evaluated using multiple quality parameters to determine its reliability. Additionally, physicochemical properties were assessed to provide insights into the molecular characteristics of the nucleoprotein. This study provides a validated structural framework for future investigations related to CCHFV nucleoprotein structure and function.

## **Materials and Methods**

### **Retrieval of CCHFV Nucleoprotein Sequence**

The amino acid sequence of Crimean-Congo haemorrhagic fever virus (CCHFV) nucleoprotein was retrieved from the National Center for Biotechnology Information (NCBI) Protein Database (<https://www.ncbi.nlm.nih.gov/protein/>). The retrieved protein sequence was used for further structural and physicochemical analyses.

### **Homology Modelling of CCHFV Nucleoprotein**

The three-dimensional structure of CCHFV nucleoprotein was predicted using the SWISS-MODEL server (<https://swissmodel.expasy.org/>), an automated platform for protein homology modelling. The experimentally determined protein structure available in the Protein Data Bank (PDB) was used as a template for generating the homology model. Template selection and model construction were performed based on sequence similarity between the target protein and available structural templates.

### **Structural Validation of the Predicted Model**

The quality of the predicted three-dimensional model was evaluated using structural validation methods. The stereochemical quality of the model was assessed through Ramachandran plot analysis, and additional model

evaluation was performed using available structure assessment parameters to determine the reliability of the predicted structure.

### Physicochemical Characterization of CCHFV Nucleoprotein

The physicochemical properties of the CCHFV nucleoprotein were analysed using the ProtParam tool available through the ExPASy server (<https://web.expasy.org/protparam/>). The analysis included evaluation of molecular weight, theoretical isoelectric point, amino acid composition, charged residue distribution, extinction coefficient, instability index, aliphatic index, and grand average of hydropathicity (GRAVY).

### Data Analysis

The obtained sequence, predicted structure, and physicochemical characteristics were analysed to characterize the molecular properties of the CCHFV nucleoprotein.

## Results

### Sequence Retrieval and Analysis of CCHFV Nucleoprotein

The amino acid sequence of the Crimean-Congo haemorrhagic fever virus (CCHFV) nucleoprotein was retrieved from the NCBI database. The selected nucleoprotein sequence (accession number: P89522) consisted of 482 amino acid residues and was used for subsequent structural and physicochemical analyses (Table 1).

Table 1. Protein sequence of CCHFV nucleoprotein obtained from NCBI

| Sr.# | Protein                                                                | Length | Protein Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|------|------------------------------------------------------------------------|--------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1    | Crimean-Congo<br>haemorrhagic fever<br>virus nucleoprotein<br>[P89522] | 482 aa | MENKIEVNNKDEMNRWFEEFKKGNGLVDFTNSYS<br>FCESVPNLDRFVFQMASATDDAQKDSIYASALVEAT<br>KFCAPIYECAWVSSTGIVKKGLEWFEKNAGTIKSWD<br>ESYTELKVDVPKIEQLTGYQQAALKWRKDIGFRVN<br>ANTAALSNKVLAEYKVPGEIVMSVKEMLSDMIRRR<br>NLILNRGGDENPRGPVSHEHVDWCREFVKGKYIMA<br>FNPPWGDINKSGRSGIALVATGLAKLAETEGKGIFD<br>EAKKTVEALNGYLDKHKDEVDRASADSMITNLLKH<br>IAKAQELYKNSSALRAQSAQIDTAFSSYYWLYKAG<br>VTPETFTPTVSQFLFELGKQPRGTTKMKKALLSTPMK<br>WGKKLYELFADDSFQQNRIYMHPAVLTAGRISEMG<br>VCFGTIPVANPDDAAQGSHTKSILNLRNTTETNNP<br>CAKTIVKLFEVQKTGFNIQDMDIVASEHLLHQSLVG<br>KQSPFQNAYNVKGNATSANII |

### Three-Dimensional Structure Modelling of CCHFV Nucleoprotein

The three-dimensional structure of the CCHFV nucleoprotein was generated through homology modelling using an available experimentally determined nucleoprotein structure as a template. The predicted structural model showed the overall three-dimensional organization of the target protein (Figure 1).

The selected template structure was identified as 4aqf.2.A, corresponding to the X-ray crystallographic structure of CCHFV nucleoprotein. The structural modelling assessment demonstrated complete query coverage and high similarity between the target sequence and template structure. The model quality parameters obtained from the structural assessment are presented in Table 2.

Table 2. Structural descriptors for evaluating model quality.

| Protein             | ID for Temp | Query (%) | Similarities (%) | GMQE |
|---------------------|-------------|-----------|------------------|------|
| CCHFV nucleoprotein | 4aqf.2.A    | 100       | 97.03            | 0.93 |

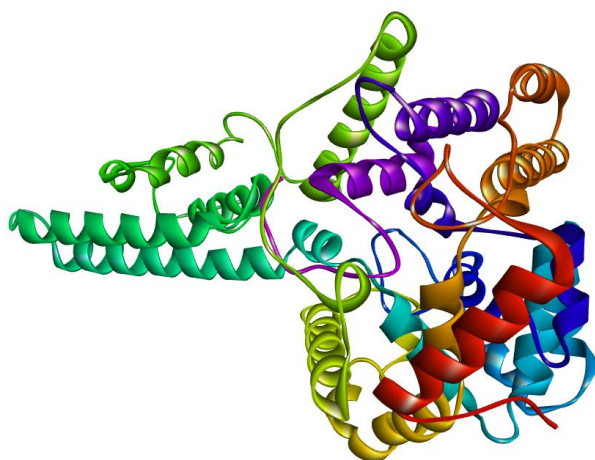


Figure 1. 3D structure of CCHFV nucleoprotein (target protein).

### Structural Validation of the Predicted Model

The quality and reliability of the predicted three-dimensional structure were evaluated using different structural validation parameters. The Ramachandran plot analysis demonstrated the distribution of amino acid residues within favoured and allowed conformational regions. The structural validation results indicated that the generated model possessed acceptable stereochemical characteristics. The Ramachandran plot and SWISS-MODEL quality assessment of the homology model are shown in Figure 2.

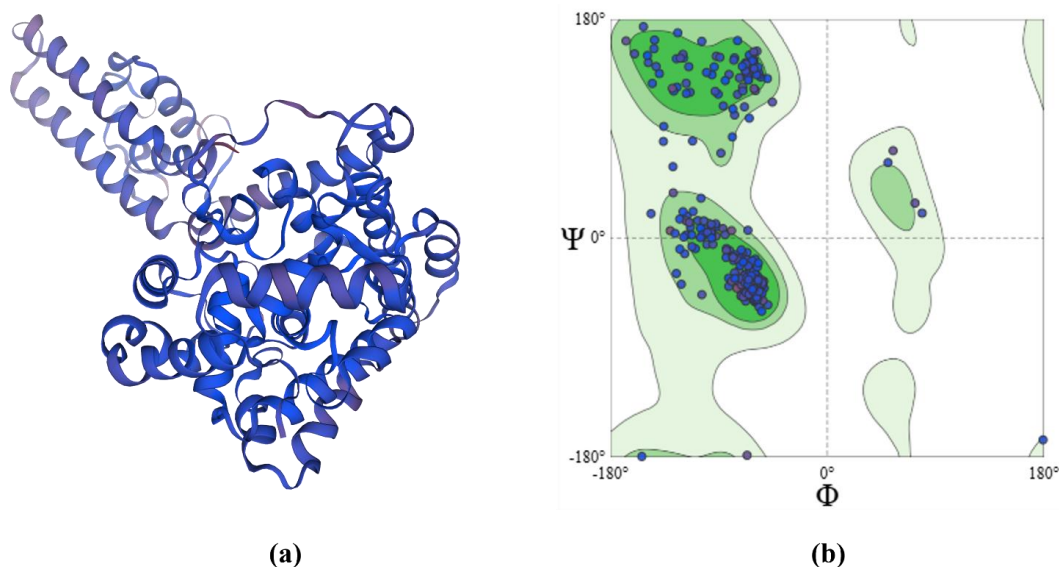


Figure 2. Structural validation of the homology-modelled three-dimensional structure of CCHFV nucleoprotein: (A) Ramachandran plot, (B) SWISS-MODEL quality assessment

Additional quality assessment parameters, including MolProbity score, clash score, Ramachandran favoured residues, Ramachandran outliers, template information, and QMEANDisCo scores, were analysed to evaluate the reliability of the predicted model.

### Physicochemical Characterization of CCHFV Nucleoprotein

The physicochemical properties of the CCHFV nucleoprotein were evaluated using the ProtParam tool available through the ExPASy server. The analysis included molecular weight, theoretical isoelectric points, charged residue composition, extinction coefficient, instability index, aliphatic index, and grand average of hydropathicity (GRAVY). The CCHFV nucleoprotein consisted of 482 amino acids with a molecular weight of 53,891.36 Da. The protein exhibited a theoretical isoelectric point of 8.47, indicating a basic nature. The numbers of negatively charged residues (Asp + Glu) and positively charged residues (Arg + Lys) were 57 and 61, respectively, resulting in an overall positive charge. The instability index, aliphatic index, and GRAVY values indicated the physicochemical characteristics of the protein (Table 3).

Table 3. Physicochemical parameters were calculated using the ProtParam tool on ExPASy

| S. No. | Physicochemical Property                | CCHFV nucleoprotein                    |
|--------|-----------------------------------------|----------------------------------------|
| i      | Number of amino acids (AAs)             | 482                                    |
| ii     | Molecular weight                        | 53891.36 Da                            |
| iii    | Theoretical isoelectric point (pI)      | 8.47 (Basic)                           |
| iv     | Negatively charged residues (Asp + Glu) | 57                                     |
| v      | Positively charged residues (Arg + Lys) | 61                                     |
| vi     | Overall charge                          | Positive (+)                           |
| vii    | Extinction coefficient                  | 71850 M <sup>-1</sup> cm <sup>-1</sup> |
| viii   | Instability index                       | 31.44 (stable)                         |
| ix     | Aliphatic index                         | 76.33                                  |
| x      | Grand average of hydropathicity (GRAVY) | -0.414                                 |

### Discussion

The present study provides a structural and physicochemical characterization of the Crimean-Congo haemorrhagic fever virus (CCHFV) nucleoprotein using bioinformatics-based approaches. The analysed nucleoprotein sequence consisted of 482 amino acid residues, corresponding to the reported size range of CCHFV nucleoprotein. The nucleoprotein is encoded by the S segment of the viral genome and plays an essential role in viral RNA encapsidation, ribonucleoprotein complex formation, and genome organization (Zivcec et al., 2016; Hawman & Feldmann, 2023). The conserved nature of this protein makes it an important subject for structural and functional investigations.

In this study, a three-dimensional structure of the CCHFV nucleoprotein was generated using homology modelling based on an experimentally resolved CCHFV nucleoprotein structure. The availability of crystallographic structures has significantly improved understanding of CCHFV nucleoprotein organization. Previous structural studies demonstrated that the nucleoprotein adopts a conserved architecture consisting of structural regions involved in RNA binding and oligomer formation, which are essential for viral genome packaging and replication (Carter et al., 2012; Wang et al., 2012). The use of a validated experimental structure as a template in the present study supports the reliability of the predicted model.

The quality of the generated model was assessed using structural validation approaches, including Ramachandran plot analysis and model quality evaluation. The predicted structure showed favourable stereochemical properties, indicating that the model represents an acceptable structural approximation of the CCHFV nucleoprotein. Similar validation strategies have been applied in previous structural studies to evaluate CCHFV nucleoprotein models and experimentally determined structures (Carter et al., 2012). The agreement between the predicted model and previously reported structural characteristics suggests that the generated model can be used for further structural investigations.

The structural organization of CCHFV nucleoprotein is closely related to its RNA-binding ability. Previous studies have demonstrated that CCHFV nucleoprotein interacts with RNA through multiple binding modes, suggesting flexibility in nucleoprotein–RNA interactions during viral genome organization (Jeeva et al., 2017). Additionally, structural analyses have shown that nucleoprotein oligomerization contributes to the formation of higher-order assemblies required for efficient genome encapsidation (Wang et al., 2012). The structural characteristics observed in the present study are consistent with these previous findings and support the conserved functional role of CCHFV nucleoprotein.

Physicochemical analysis of nucleoprotein revealed important molecular characteristics, including molecular weight, theoretical isoelectric point, charge distribution, instability index, aliphatic index, and hydropathicity. The basic nature of the protein observed in this study is consistent with the expected properties of RNA-binding proteins, where positively charged residues may contribute to interactions with negatively charged RNA molecules. Similar relationships between nucleoprotein charge properties and RNA-binding activity have been described in structural investigations of CCHFV nucleoprotein (Carter et al., 2012; Jeeva et al., 2017).

The stability-related parameters obtained from physicochemical analysis provide additional information regarding the molecular behaviour of the protein. The calculated instability index indicated a stable protein configuration, supporting the suitability of the predicted three-dimensional structure. Computational characterization of viral proteins using sequence-derived physicochemical parameters has been widely applied as a complementary approach to experimental structural studies (Zivcec et al., 2016).

Overall, the findings of this study demonstrate that the predicted CCHFV nucleoprotein structure exhibits reliable structural characteristics and physicochemical properties consistent with previously reported experimental studies. The validated structural model provides additional insight into CCHFV nucleoprotein organization and establishes a foundation for future investigations focusing on the molecular function and structural biology of this important viral protein.

## **Conclusion**

The present study provides a comprehensive structural and physicochemical characterization of the CCHFV nucleoprotein using bioinformatics-based approaches. The nucleoprotein sequence was successfully analysed, and a reliable three-dimensional model was generated through homology modelling and evaluated using structural validation parameters. The predicted model exhibited favourable structural quality, while physicochemical analysis revealed important molecular characteristics related to protein composition, charge distribution, stability, and hydropathic properties. The obtained findings support the conserved structural features of CCHFV nucleoprotein and enhance understanding of its molecular organization. Overall, this validated structural model provides a valuable framework for future investigations into the structure and function of CCHFV nucleoprotein and contributes to further studies on the molecular biology of this important viral pathogen.

## **Scientific Ethics Declaration**

\* The authors declare that the scientific ethical and legal responsibility of this article belongs to the authors. No human or animal experimental procedures were conducted in this review article; therefore, ethics committee approval was not required.

## **Conflict of Interest**

\* The authors declare that they have no conflicts of interest.

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