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Review Article

Structures and Functions of Coronavirus Proteins: Molecular Modeling of Viral Nucleoprotein -

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ABSTRACT

Background: In recent times, several life-threatening viruses have emerged and coronaviruses (CoVs) are one of these. They have infected a variety of human and animal hosts, causing illnesses that range from mostly upper respiratory tract infections in human, encephalitis and demyelination in animals, which can be fatal. They have been responsible for causing significant human and animal mortality, in addition to raising serious public health concerns worldwide. Two novel viruses Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) were implicated to be responsible for severe acute illness in recent times. Unfortunately, no effective treatments against these viruses are available.

Results: There is an urgent need to develop new strategies to prevent or control coronavirus infections, and understanding the biology, replication, and pathogenesis of these viruses. Therefore, in this review we have described CoVs proteins structure and functions with experimental and computational studies.

Conclusion: This information may lead to a better understanding of the function of CoV proteins in the virus replication and transcription mechanism and to develop pioneering antivirals.

Keywords: Coronaviruses; SARS-CoV; MERS-CoV; CoVs protein; Molecular Modeling Methods.

INTRODUCTION

Coronaviruses (CoVs) are a diverse family of viruses. They cause a variety of diseases in mammals and birds ranging from enteritis in cows and pigs and upper respiratory disease in chickens to potentially lethal human respiratory infections. Severe acute respiratory syndrome (SARS), the first identified in 2002 and diagnosed in Southern China, occurred from a human CoV [1]. Then, exactly 10 years after the SARS-CoV emergence, a new emerging Coronavirus named Middle East Respiratory Syndrome (MERS-CoV) has infected people with a high mortality rate of nearly 50% in the Middle East. 10 March 2016 the World Health Organization (WHO) global case count for MERS was 1,651 laboratory-confirmed cases, including at least 590 deaths (case fatality rate 36%) since the first cases were reported in September 2012 [2]. The murine coronavirus, Mouse Hepatitis Virus (MHV), which is closely related to SARS and MERS-CoV, has long served as a model for study of both the molecular biology and pathogenesis of members of this viral family.

These viruses infect a variety of human and animal host cells, and also carry out their infection and replication. Also, many proteins have most important role in the replication mechanism, although that role is, as yet, poorly defined. In this case, it is necessary to know the definition of these proteins in terms of this mechanism. Therefore, this review explains the classification and genome organization of coronaviruses and then it summarizes the structure and function of these proteins in the replication and transcription mechanism from a more biophysical point of view by using molecular modeling methods. This information is expected to assist elucidation of the replication and transcription mechanism of the CoV.

Classification of coronaviruses

The classification of Coronaviruses has been based on genomic organization, similarities in genomic sequence, antigenic properties of viral proteins, replication strategies, and structural characteristics of virions, pathogenic, cytopathogenic and physicochemical properties [3]. The Coronaviruses (CoVs) are species of virus belonging to the Nidovirales order, which includes *Coronaviridae*, *Arteriviridae*, *Roniviridae* and *Mesoniviridae* families [4]. The *Coronaviridae* family is the largest one of the four families, by its genomic sizes of *coronaviridae* range from 26 to 32 kb [5]. *Coronaviridae* virus family subdivided into two subfamilies, *coronavirinae* and *torovirinae* [6]. It is now divided into four genera, Alpha coronavirus, Beta coronavirus, Gamma coronavirus and Delta coronavirus (Figure 1).

Alpha coronaviruses type 1 species are classified feline FCoV, FECV (Feline Enteric Coronavirus) and FIPV (Feline Infectious Peritonitis Virus), the porcine TGEV (Transmissible Gastro-Enteritis Virus), Porcine PEDV (Epidemic Diarrhea Virus), PRCoV (Porcine Respiratory Coronavirus) and the canine CCoV. Alpha coronaviruses also compromise human CoVs such as HCoV-229E and HCoV-NL63, but various bat Coronaviruses. Beta coronaviruses also infect a wide range of mammals, with species such as mice, human with SARS-CoV, HCoV-OC43, HCoV-HKU1, and MERS-CoV, Murine coronavirus (MHV) and Bovine Coronavirus (BCoV). Gamma coronaviruses are specific of birds, with one exception of a beluga whale Coronavirus. The delta coronavirus genus was created in 2012 and regroups various (HKU11, HKU12, HKU13) Coronavirus from mammals to birds [7].

Genome structure of coronaviruses

Coronaviruses encode five structural proteins in their genomes. These are the Spike (S), Membrane (M), Envelope (E) glycoproteins,

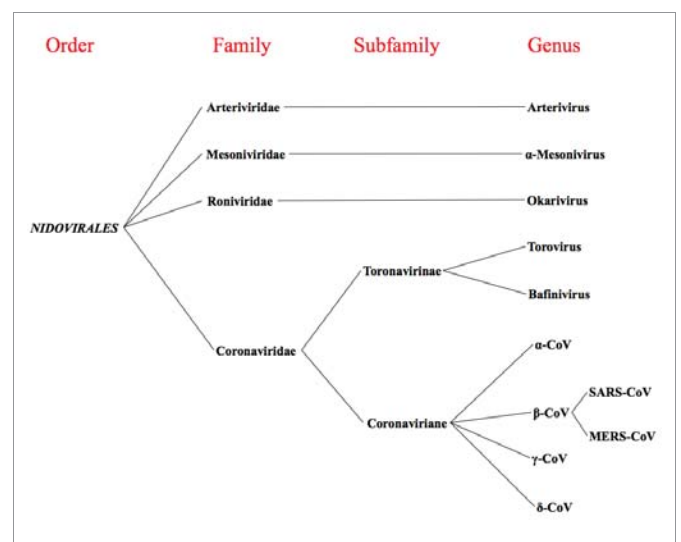


Figure 1: Classification of coronaviruses.

The first OC43 strain of the human beta coronavirus family was characterized in 1967 as agents of human respiratory diseases [8]. Until 2002, the new disease identified, Severe Acute Respiratory Syndrome (SARS), is caused by a human CoV that emerged in Southern China and Hong Kong. Following the SARS epidemic, two new human coronaviruses named HCoV-HKU1 and HCoV-NL63 have been identified [9-10].



Hemagglutinin Esterase (HE) and Nucleocapsid (N) protein, (Figure 2). All envelope proteins and N protein is present in all virions but HE is only present in some beta coronaviruses [11]. In addition to that, it is thought the virus particles are huddled together owing to interaction between these proteins [12,13].

S Glycoproteins: S Glycoproteins are located outside the virion and give the virion the typical shape. The S proteins form homotrimers, which allow the formation of sun-like morphologies that give the name of Coronaviruses [15-17]. S proteins bind to the virion membrane via the C-terminal transmembrane regions and they also interact with M proteins [18]. Virions can be bound to specific surface receptors in the plasma membrane of the host cell via the N-terminus of the S proteins [19].

M Glycoproteins: M Glycoproteins have three transmembrane regions. M proteins are glycosylated in the Golgi apparatus [20-22]. This modification of the M protein is crucial for the virion to fuse into the cell and to make protein antigenic [23-25]. The M protein plays a key role in regenerating virions in the cell. N protein forms a complex by binding to genomic RNA and M protein triggers the formation of interacting virions in this endoplasmic reticulum-Golgi apparatus intermediate compartment (ERGIC) with this complex [20,26,27].

E Glycoproteins: E Glycoproteins are small proteins that are composed of approximately 76 to 109 amino acids. About 30 amino acids in the N-terminus of the E proteins allow attachment to the membrane of viruses [28]. In addition, coronavirus E proteins play a critical role in the assembly and morphogenesis of virions within the cell. In one study coronavirus E and M proteins were expressed together with mammalian expression vectors to form virus-like structures within the cell [29-31]. In another study, there was a significant decrease in the ability of the recombinant mouse hepatitis virus (MHV) and SARS viruses to elicit E protein expression in the genome to support this status [32,33].

N Proteins: N proteins are phosphoproteins that are capable of binding to helix and have flexible structure of viral genomic RNA. It plays an important role in virion structure, replication and transcription of coronaviruses, because the N protein localizes in both the replication/transcriptional region of the coronaviruses and the ERGIC region where the virus is collected [26-34].

Introduction of Virus into the Cell, Replication and Transcription

The replication of coronaviruses occurs in host cell cytoplasm. The viruses primarily bind to the receptor on the cell surface via the spike (S) protein. When S protein is bound to the receptor, a conformational structure occurs in the structure and the process of entry into the virus cell begins [35,36]. This process with endocytosis is dependant of pH through the receptor [37-40]. After entering the cytoplasm, the virus particle releases the RNA genome. This genome is a single-stranded, non-segmented RNA virus with the largest known RNA genome (gRNA), which is approximately 26-32 kb (Figure 3) [41-44]. The genome consists of seven genes. It is organized into 5' non-structural protein coding regions comprising the replicase genes (gene 1), which are two-thirds of the genome, and 3' structural and nonessential accessory protein coding regions comprising the gene 2-7[13,45].

The replicase gene 1 products are encoded two very large open reading frames ORF1a and 1b, which are translated into two large polypeptides pp1a and pp1b, which are synthesized directly from the 5' two-thirds of the genomic RNA of CoV. After synthesis of these proteins, consisting of 16 units, non-structural protein (nsp1

to nsp16) is converted with the contribution of viral proteases pp1a and pp1b [46-48]. These 16 proteins form Double-Membrane Vesicles (DMV). At the same time, this DMV is virus Replication and Transcription Complex (RTC) [49,50]. These nsp proteins, especially non-structural protein3 (nsp3), have an important role in the virion structure, the replication and transcription of CoV [51-53].

Genes 2 to 7 are translated from sub genomic mRNA. Sub genomics RNAs encode the major viral Structural proteins (S), Envelope protein (E), Membrane protein (M), Nucleocapsid protein (N), and the accessory proteins, which are essential for virus-cell-receptor binding. The newly structural synthesized proteins are released into the endoplasmic reticulum. All of these proteins, along with the N protein, are linked to the viral genomic RNA and localized in the ERGIC region [34,49,54,55] (Figure 4). Although, N protein is known to be necessary for coronavirus replication, the specific role that this protein plays in this process remains unknown. But, many studies suggest that N protein interaction with nsp3 plays a critical role in the virus replication early in infection. Therefore, the next section gives a detail information about determination of structure N and interaction mechanism of N protein and nsp3 protein.

Clarification of Structure MHV N protein and Determinants of Interaction with nsp3 Protein via the Molecular Modeling Methods

The nucleocapsid (N) protein plays an essential role in the virus structure, the replication and transcription of CoV via interactions

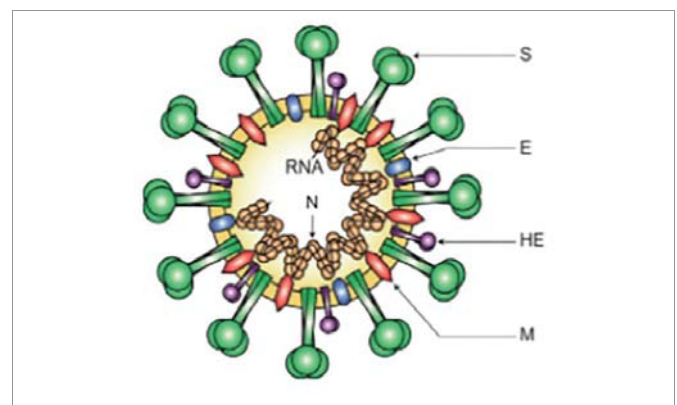


Figure 2: Coronavirus virion structure shown with structural proteins. N: Nucleocapsid protein; S: Spike protein, M: Membrane protein, HE: Hemagglutinin-Esterase and E: Envelope protein [14].

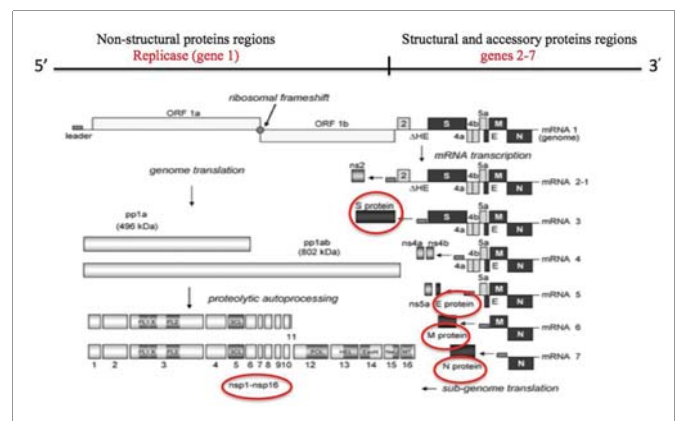


Figure 3: Mouse Hepatitis Virus (MHV) genome organization [56].



with the large positive-strand RNA viral genome. It is also shown that it plays a significant role in the pathogenesis of mouse hepatitis virus (MHV) and human CoV-induced diseases (SARS-CoV, MERS-CoV) which is a major determinant of virulence [34,57-59]. Therefore, it is necessary to determinate structure of N to understand the replication and transcription mechanism. Thus, this section gives information about the structure and function of MHV N protein.

The MHV N protein contains two structurally independent RNA binding domains which are called as N-terminal domain (NTD) and C-terminal (CTD) dimerization domain linked by a charged linker region which is rich in Ser/Arg (SR-rich linker) (Figure 5). The NTD domain (amino acids range 60-194) and SR-rich region (amino acids range 195-230) are known as they play an important role in CoV, both in viral replication and transcription [60,61]. For example; NMR chemical shift perturbation studies [62] reveal that the regions of the SR-rich and NTD in MHV N protein interact with MHV nsp3. They, which are N and nsp3 proteins, also mapped basic determinants of this high affinity binding to the SR region of N and acidic $\alpha 2$ helix of nsp3. Moreover, Masters PS, et al. [58] found that there was a correlation between the N-nsp3 interaction and the ability of N protein to stimulate the infectivity of MHV gRNA. These results confirm the critical nature of N and nsp3 interaction and provide further support for its proposed role in the initiation of coronavirus infection. Nicolas E. Grosseohme, et al. [62] created 3D structure of NTD MHV N protein by using X-ray method (PDB code: 3HD4). This structure includes only NTD domain (53-194) but unfortunately, N-nsp3 interaction mechanisms in the virus replication plays an important role in three-dimensional structure of the SR-rich region (amino acids range 195-230), which cannot be illuminated by the experimental methods [62]. In this case, Tok research group [63] generated a 3D structural model of SR-rich region MHV N protein with homology modeling. This method is based on matching the amino acid sequence of the protein to be modeled with other amino acid sequences in the data banks. Amino acid sequence analysis of the N proteins from a variety of coronavirus (SARS-CoV and Human-CoV OC43) strains suggest that a highly conserved structure within NTD domain and SR-rich region (Figure 6) and the 3D structure of the protein of SARS-coronaviruses (pdb code: 1SSK) and Human-CoV OC43 (pdb code: 4J3K) are available (Figure 7). They selected these proteins as template proteins for homology model of the SR-rich region MHV N protein. After all, this model protein has 53-230 amino acids site, which included NTD and SR-rich region (Figure 8). The MHV nsp3 protein 3D structure is available (pdb code: 2M0A) (Figure 8) [62], therefore there was no need to create a homology model, thus this crystal structure is used for docking. Then, this research group has studied protein-protein docking with N and nsp3 complex in order to illuminate amino acids, which play an important role in virus replication, and reveal replication mechanism of those functions in the epitope portions. As a result, binding site of N proteins Arg 125, Tyr 127 and Tyr 190 residues overlap with the experimental study [62,64,65]. At the same time, different residues (Lys 113, Glu 173) were also predicted for this binding site (Figure 9). On the other hand, mutation analyses were done on these residues. The results reveal that these mutations cause destabilization effect of protein structures and the interaction between this protein and the nsp3 protein is disrupted. Consequently, these residues have an important role in virus replication.

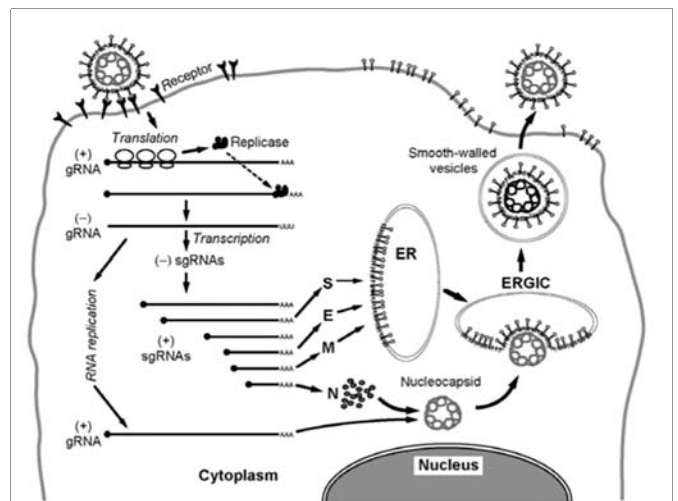


Figure 4: Coronavirus' life cycle [13].

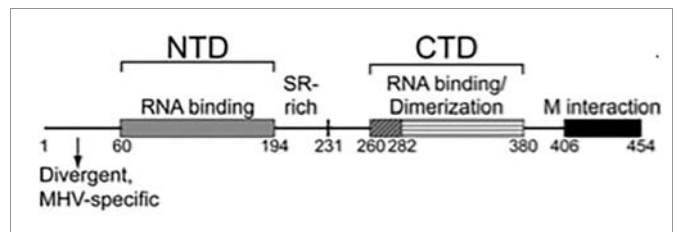


Figure 5: The domain regions of N protein [62].

MHV-nprotein	RSFVQDERAGSSSVNAGDGLAKTTFWADGTEPRHGRHGRHGFQSTATQRRG	60
SARS-CoV-nprotein	RS-----DSGQ-----SQRASRRTFTGPTSTDRHGRHGRHGF-----EQRFGQ	65
HumanOC43-nprotein	RS-----DSGQ-----SQRASRRTFTGPTSTDRHGRHGRHGF-----EQRFGQ	65
MHV-nprotein	SVVVWFVFLITQFQKRETFARSDQFLIANGIAPRSGVYVHNSAFATYDQGG	120
SARS-CoV-nprotein	LFVHVAWFTALQHG-----KEELFPHGQGFVITRHSRGGQIYTKA-----TAVYVGGGQAK	163
HumanOC43-nprotein	LFVHVAWFTALQHG-----KEELFPHGQGFVITRHSRGGQIYTKA-----TAVYVGGGQAK	163
MHV-nprotein	QLLFWYFTLGTQPRASGIVGIIQVYVWAGADTETWEDIVEDRDSREALITFPA	180
SARS-CoV-nprotein	ELSPWYFTLGTQPEALFVQANSLIHWATEGALITFDRIOTRHSRDAVYVGLP	183
HumanOC43-nprotein	ELSPWYFTLGTQPEALFVQANSLIHWATEGALITFDRIOTRHSRDAVYVGLP	183
MHV-nprotein	PCVTLPGQFVYVAGAFAS-----RGRSGRQPHRARSQNGVQASTVYDRAEIAA	239
SARS-CoV-nprotein	QVTLVPGQFVYVAGAFAS-----RGRSGRQPHRARSQNGVQASTVYDRAEIAA	222
HumanOC43-nprotein	QVTLVPGQFVYVAGAFAS-----RGRSGRQPHRARSQNGVQASTVYDRAEIAA	222
MHV-nprotein	LVLAALGDA-----GQFV-----QVYVQKAEVQGLIHFPHQRTFPHGQVQCCGDSGSP	284
SARS-CoV-nprotein	LLSLNGLDEKFRVHGGQGGQVYVYKAAKARFPHQRTFPHGQVQCCGDSGSP	282
HumanOC43-nprotein	LLSLNGLDEKFRVHGGQGGQVYVYKAAKARFPHQRTFPHGQVQCCGDSGSP	282
MHV-nprotein	---HGGSEKLGTSDFVFLIHLAFLVYVAFVFGKELVHNSGADSTYGVLEQ	351
SARS-CoV-nprotein	TQVHVGQGLIHQDTVSRVFLIHLAFLVYVAFVFGKELVHNSGADSTYGVLEQ	333
HumanOC43-nprotein	TQVHVGQGLIHQDTVSRVFLIHLAFLVYVAFVFGKELVHNSGADSTYGVLEQ	333
MHV-nprotein	YEGVYFESTLFCFETLRVLDLHAYVGGQGGV-----SFFPHRQSPQAG	401
SARS-CoV-nprotein	YEGVYFESTLFCFETLRVLDLHAYVGGQGGV-----SFFPHRQSPQAG	391
HumanOC43-nprotein	YEGVYFESTLFCFETLRVLDLHAYVGGQGGV-----SFFPHRQSPQAG	391
MHV-nprotein	EEKEDVDVQVAPVSDGVVYKSLTFRKLLAQILDGVVQDLEDGSPV	454
SARS-CoV-nprotein	---TVTL---FAAD-NDGFVQLGHS-----RGSAGDTGA-----	422
HumanOC43-nprotein	---TVTL---FAAD-NDGFVQLGHS-----RGSAGDTGA-----	422

Figure 6: Multiple alignments of N proteins in different coronaviruses. These alignments were calculated on the Clustal Omega server (<http://www.ebi.ac.uk/Tools/msa/clustalo/>).

CONCLUSIONS AND FUTURE DIRECTIONS

Coronaviruses (CoVs) are a diverse family of viruses that interact at multiple levels with components of host cells taking this advantage of some of the cellular machineries for replication and proliferation. Various are known about the molecular biology of CoVs but more information is needed to learn. For example, many of the non-structural and accessory proteins encoded by these viruses remain uncharacterized with no known function, and it will be important to identify mechanisms of action for these proteins as well as defining their role in viral replication and pathogenesis. The challenge now

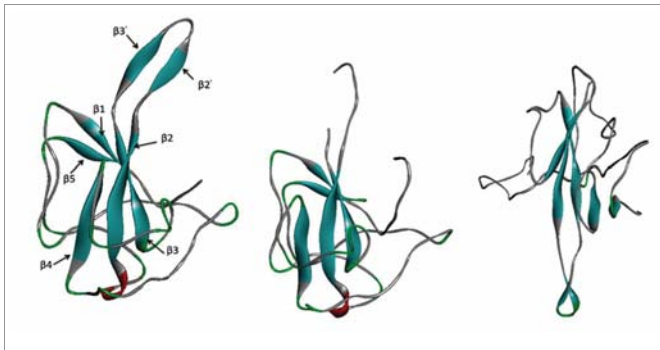


Figure 7: 3D structures of MHV N protein (3HD4) (left side), SARS-CoV (1SSK) (middle side) and Human-CoV OC43 (4J3K) (right side).

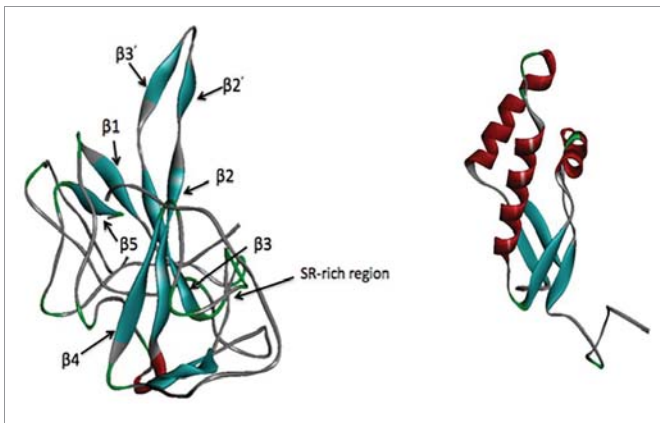


Figure 8: 3D model structure of MHV N-SR rich region (left side) and the crystal structure of nsp3 protein (2M0A) (right side).

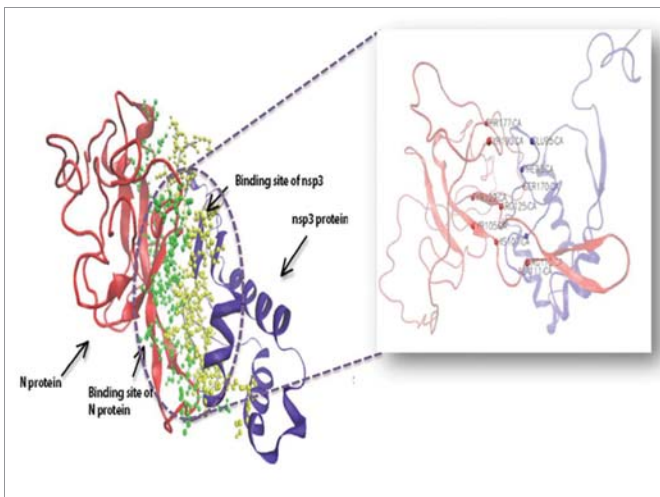


Figure 9: Protein-Protein interactions between N and nsp3 protein.

is to incorporate advance techniques in the investigative efforts done to understand further the biology of CoVs. However, these experimental studies made quite precise, to be dangerous and costly, most importantly 3rd degree with these curity level laboratories, it remains limited around the world. Lately, especially in the United States, Taiwan, Switzerland, European Union member countries, including Computational Molecular Modeling Studies are effective in removing this deficit, and often have an increased use (Bisson, WH, 2012). Due to work and difficulty are developed vaccines that

naturally cover yet have no effective medication that has resulted. Developing technology is going to be getting important insight about structure of CoVs protein to define the mechanism of how protein cause disease and understanding the protein-protein and protein-RNA interaction will significantly improve our ability to design vaccines. In the meantime, molecular modeling methods provide important solutions to the struggle.

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