Scientific Journal of Biology

Research Article

Structure Model Analysis of the Effects of Mutations in the Phosphorylation Sites of SARS-CoV-2 Encoded Nucleocapsid Protein -

Pierre Limtung and HY Lim Tung*

Peptide and Protein Chemistry Research Laboratory, Nacbraht Biomedical Research Institute, New York City (Astoria), New York 11106, USA

***Address for Correspondence:** HY Lim Tung, Peptide and Protein Chemistry Research Laboratory, Nacbraht Biomedical Research Institute, 3164 21st Street, Suite 122, Astoria (NYC), NY 11106, USA, Tel: +332-201-7161; E-mail: hyltung2010@nacbrahtbiomedresins.org

Submitted: 23 October 2020; **Approved:** 28 October 2020; **Published:** 30 October 2020

Cite this article: Limtung P, Lim Tung HY. Structure Model Analysis of the Effects of Mutations in the Phosphorylation Sites of SARS-CoV-2 Encoded Nucleocapsid Protein. Sci J Biol. 2020 Oct 30;3(1): 023-030. doi: 10.37871/sjb.id19

Abstract

Mutations in several phosphorylation sites within the phosphorylation rich domain of SARS-COV-2 Nucleocapsid protein (NCp), including serines 186, 197 and 202, and adjacent arginine 203 and glycine 204 have been described and have been proposed to prevent the binding and sequestration of NCp by Protein 14-3-3. Structure modeling and thermodynamic calculation show that mutations of phosphorylation sites, phospho-serines 186, 197 and 202 to phenylalanine, leucine and asparagine, and phosphorylation recognition sites, arginine/glycine 203/204 to lysine/arginine or lysine/threonine resulted in significant destabilization of the NCp-14-3-3 complex by causing a decrease in Stability Energy ($\Delta G_{\text{stability energy}}$) and Binding Energy ($\Delta\Delta G_{\text{binding energy}}$). These results evidenced that mutations in NCp underlie a mechanism to bypass sequestration by Protein 14-3-3 which would result in enhanced dimerization of NCp, and replication, transcription and packaging of the SARS-COV-2 genome.

INTRODUCTION

SARS-COV-2 Nucleocapsid protein (NCp) plays an essential role as a co-factor in the initiation and control of the replication, transcription and packaging of the SARS-COV-2 genome [1-6]. Dimerization of SARS-COV-2 Nucleocapsid protein (NCp) is a prerequisite step in the replication, transcription and packaging of the SARS-COV-2 genome. Oligomerization of NCp is also necessary for the packaging of SARS-COV-2 genome [7-12]. Phosphorylation of NCp has been proposed to play an important role in the control of NCp functions [13-17]. A cellular response mechanism for preventing dimerization of NCp involving phosphorylation dependent sequestration of monomeric NCp by Protein 14-3-3 has been proposed [13]. Inhibiting the dimerization and oligomerization of NCp is an attractive way to control SARS-COV-2 viability, infection and virulence [13,18].

Phosphorylation of serines 197 and 206 of NCp by C-TAK-1 has been proposed to constitute the formation of the binding and sequestration sites (RNpSTP and RGTpSP) that are located in the linker region of NCp for Protein 14-3-3 [13,18]. Other phosphorylations sites, including serines 186 and 202, and threonines 198 and 205 are also present in the phosphorylation rich domain of NCp [13,18]. Serines 186, 197 and 202 become mutated to phenylalanine, leucine and asparagine in SARS-COV-2 strains/sub-strains from Iran, Spain and India respectively. Arginine 203 and glycine 204 are mutated to lysine and arginine or lysine and threonine in SARS-COV-2 strains/sub-strains from Israel, Italy, Poland, Bangladesh, Greece and Czech Republic [13]. The significance of these mutations is not known. Here, we show by computational structure model analysis, and thermodynamic calculation that mutations of the phosphorylation sites, phospho-serines 186, 197 and 202, and the adjacent amino acids, arginine 203 and glycine 204 caused decreases in the Stability Energy ($\Delta G_{\text{stability energy}}$) and Binding Energy ($\Delta\Delta G_{\text{binding energy}}$) of the NCp-Protein 14-3-3 complex. It is submitted that cells infected with SARS-COV-2 possess a cellular response mechanism for the binding and sequestration of NCp by Protein 14-3-3 involving multi-sites phosphorylation by a variety of cellular protein kinases. In counterpart, SARS-COV-2 has evolved to evade the cellular response mechanism through mutations of at least 3 phosphorylation sites, serines 186, 197 and 202 and adjacent phosphorylation recognition sites (RNpSTP and RGTpSP), arginine 203 and glycine204.

METHODS

The de novo rendering of the structure of dephospho-NCp using the Quark Program pursuant to et al. [19,20] was as described previously [13,18]. Phosphorylation of SARS-COV-2 Nucleocapsid protein (NCp) was performed with FoldX using the Build Model Program pursuant to Guerois et al. and Schymkowitz, et al. [21,22]. Mutation of amino acid residues within NCp was also performed with FoldX using the Build Model Program pursuant Guerois, et al. and Schymkowitz et al. [21,22]. Docking experiments to identify the binding and sequestration of NCp by Protein 14-3-3 and dimerization of NCp were performed using the ZDOCK program pursuant to Pierce, et al. [23]. NCps rendered in this work and Protein 14-3-3 (1YZ5) based on the structure determination of Benzinger, et al. [24] were analyzed and visualized by the CCP4 Molecular Graphics Program Version 2.10.11 as described by Mc Nicolas, et al [25]. Realistic rendering of the structure of NCps were performed with the ZMM Molecular Modeling Program as described by Garden and Zhorov [26].

Determination and calculation of Stability Energy ($\Delta G_{\text{stability energy}}$) of the protein complexes and non-complexed proteins was performed using the Stability Program of FoldX as described by Guerois et al. and Schymkowitz, et al. [21,22]. Binding Energy ($\Delta\Delta G_{\text{binding energy}}$) of protein complex was determined and calculated using the Analyze Complex Program of FoldX as described by Guerois et al. and Schymkowitz, et al. [21,22]. Binding Energy Difference ($\Delta\Delta\Delta G_{\text{binding energy difference}}$) between phospho-NCp-Protein 14-3-3 complex and phospho-NCp mutants-Protein 14-3-3 complex was calculated pursuant to Teng et al. and Nishi, et al. [27-29] from the equation: $\Delta\Delta\Delta G_{\text{binding energy difference}} = \Delta\Delta G_{\text{binding energy}}$ (binding energy of phospho-NCp-14-3-3 complex) - $\Delta\Delta G_{\text{binding energy}}$ (binding energy of phospho-NCp mutants-14-3-3 complex.

RESULTS

The consequences of mutations in the phosphorylation sites of NCp were determined with respect to binding and sequestration of NCp by Protein 14-3-3. Figures 1 and 2 summarize the dockings of phospho-serine 186-NCp and phenylalanine 186-NCp mutant with Protein 14-3-3. Both phospho-serine 186-NCp and phenylalanine 186-NCp mutant form complexes with Protein 14-3-3 with very subtle conformational change. However, the calculated Stability Energy ($\Delta G_{\text{stability energy}}$) and Binding Energy ($\Delta\Delta G_{\text{binding energy}}$) of phospho-serine 186-NCp-Protein 14-3-3 were ~605 Kcal/mol and ~215 Kcal/mol respectively while the calculated Stability Energy ($\Delta G_{\text{stability energy}}$) and Binding Energy ($\Delta\Delta G$) of phenylalanine 186-NCp mutant-Protein 14-3-3 complex was ~597 Kcal/mol and ~208 respectively. These results suggest that mutation of serine 186 of NCp to phenylalanine was accompanied by destabilization of the NCp-Protein 14-3-3 complex and reduction of the binding affinity between NCp and protein 14-3-3. The Binding Energy Difference ($\Delta\Delta\Delta G_{\text{binding energy difference}}$) between phospho-serine 186 NCp-Protein 14-3-3 complex and phenylalanine 186-NCp mutant-Protein 14-3-3 complex was calculated to be ~ -7 Kcal/mol. Pursuant to Teng et al. and Nishi, et al. [27-29], negative Binding Energy Difference is thermodynamic evidence of destabilization and lowering of binding

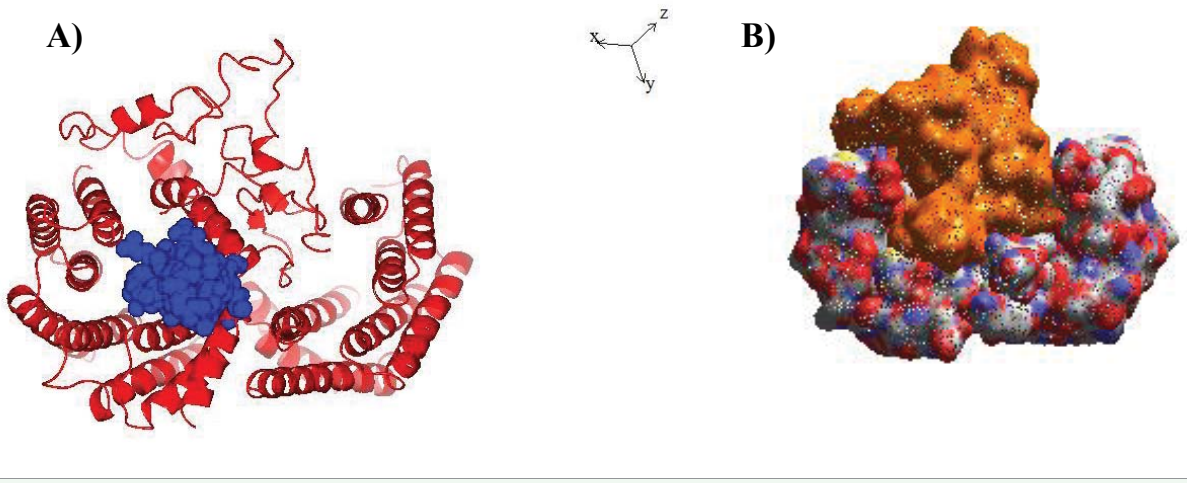

Figure 1: A: Ribbon structure (red) of phospho-serine 186-SARS-COV-2 Nucleocapsid protein (NCp) and protein 14-3-3 complex, rendered as described in method section. Phosphorylation rich domain is shown as blue spheres.
B: Realistic rendering of phospho-serine 186-SARS-COV-2 Nucleocapsid protein (NCp) (yellow) and protein 14-3-3 pre-complex (red and blue).

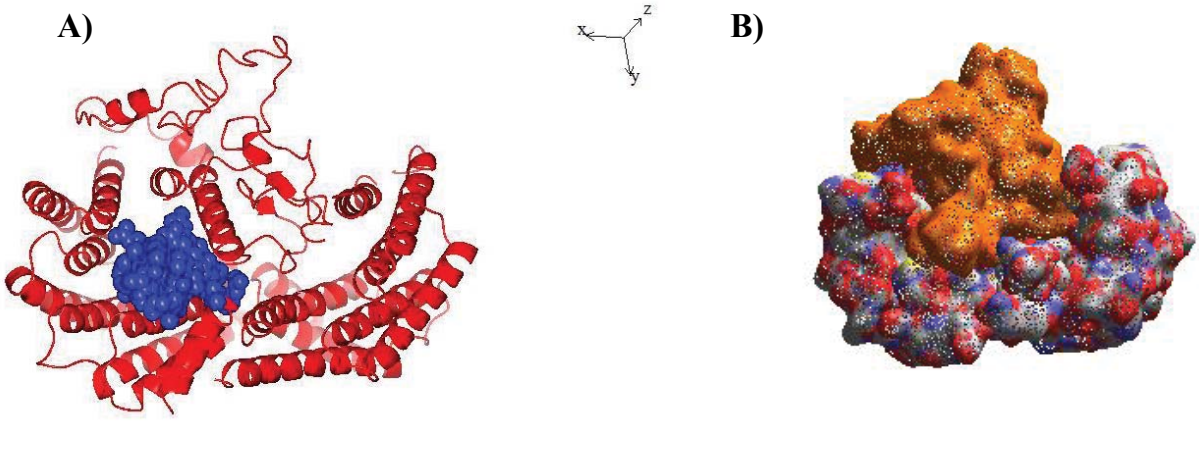

Figure 2: A: Ribbon structure (red) of S186F-SARS-COV-2 Nucleocapsid protein (NCp) and protein 14-3-3 complex, rendered as described in method section. Blue spheres show the phosphorylation rich domain.
B: Realistic rendering of S186F SARS-COV-2 Nucleocapsid protein (NCp (yellow) and protein 14-3-3 (red and blue) complex.

efficiency.

Figures 3 and 4 are summaries of the docking of phospho-serine 197-NCp and leucine 197-NCp mutant with Protein 14-3-3. Complexes between both phospho-serine 197-NCp and leucine 197-NCp mutant and Protein 14-3-3 are formed, and there was significant change in the conformation of the NCp mutant molecule which was reflected by a large difference in Stability Energy($\Delta G_{\text{stability energy}}$) and Binding Energy ($\Delta\Delta G$) of the leucine 197-NCp mutant-Protein 14-3-3 complex . The calculated Stability Energy ($\Delta G_{\text{stability energy}}$) and Binding Energy ($\Delta\Delta G$) of phospho-serine 197-NCp-Protein 14-3-3 were calculated to be ~639 Kcal/mol and ~244 Kcal/mol respectively while the calculated Stability Energy ($\Delta G_{\text{stability energy}}$) and Binding Energy ($\Delta\Delta G_{\text{binding energy}}$) of leucine 197-NCp mutant-Protein 14-3-3 complex was ~559 Kcal/mol and ~170 Kcal/mol respectively. The Binding Energy Difference ($\Delta\Delta\Delta G_{\text{binding energy difference}}$) between phospho-serines197 NCp-Protein 14-3-3 complex and leucine 197-NCp mutant-Protein 14-3-3 complex was calculated to be ~ -74 Kcal/mol. These results are consistent with the conclusion that mutation of serine 197 of NCp to leucine was accompanied by destabilization of the NCp-Protein 14-3-3 complex and reduction of the binding affinity between NCp and protein 14-3-3.

Docking experiments between phospho-serine 202-NCp and asparagine 202-NCp mutant and Protein 14-3-3 are depicted in figures 5 and 6. There was significant change in the conformation of the NCp mutant molecule. The calculated Stability Energy ($\Delta G_{\text{stability energy}}$) of phospho-serine 197-NCp-Protein 14-3-3 and asparagine-NCp mutant-Protein 14-3-3 complex were ~574 Kcal/mol and ~499 Kcal/mol respectively while the calculated Binding Energy ($\Delta\Delta G_{\text{binding energy}}$) of phospho-serine 197-NCp-Protein 14-3-3 and asparagine-NCp mutant-Protein 14-3-3 complex were ~184 Kcal/mol and ~112 Kcal/mol respectively. The Binding Energy Difference ($\Delta\Delta\Delta G_{\text{binding energy difference}}$) between phospho-serine 202 NCp-Protein 14-3-3 complex and leucine asparagine 202-NCp mutant-Protein 14-3-3 complex was calculated to be ~ -72 Kcal/mol. These results are consistent with the conclusion that mutation of serine 202 of NCp to asparagine was accompanied by destabilization of the NCp-Protein 14-3-3 complex and reduction of the binding affinity between NCp and protein 14-3-3.

Table 1 summarizes the thermodynamic calculations of various

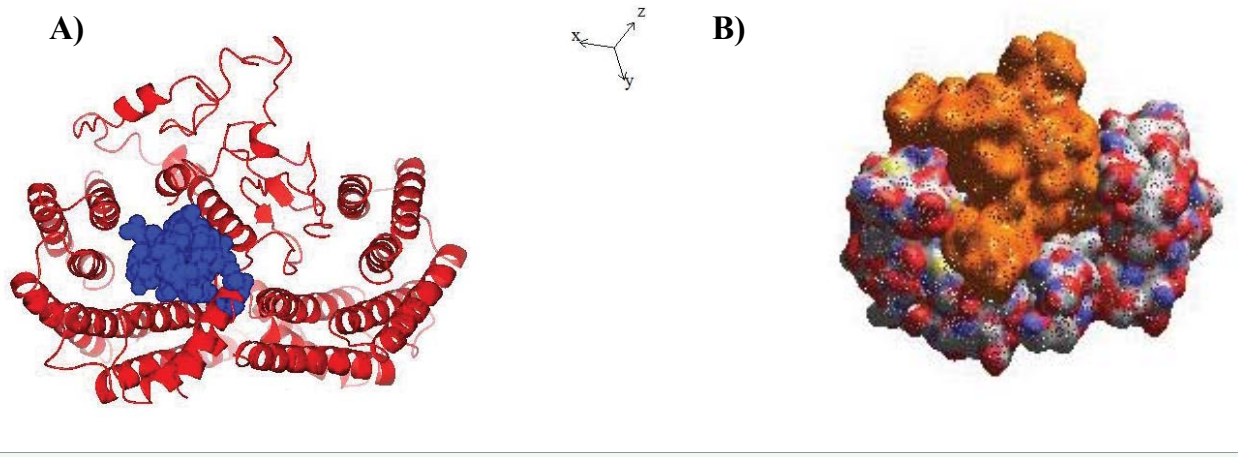

Figure 3: A: Ribbon structure (red) of phospho-serine 197-SARS-COV-2 Nucleocapsid protein (NCp) and protein 14-3-3 complex, rendered as described in method section. Phosphorylation rich domain is shown as blue spheres.
B: Realistic rendering of phospho-serine 186-SARS-COV-2 Nucleocapsid protein (NCp) (yellow) and protein 14-3-3 pre-complex (Red and Blue).

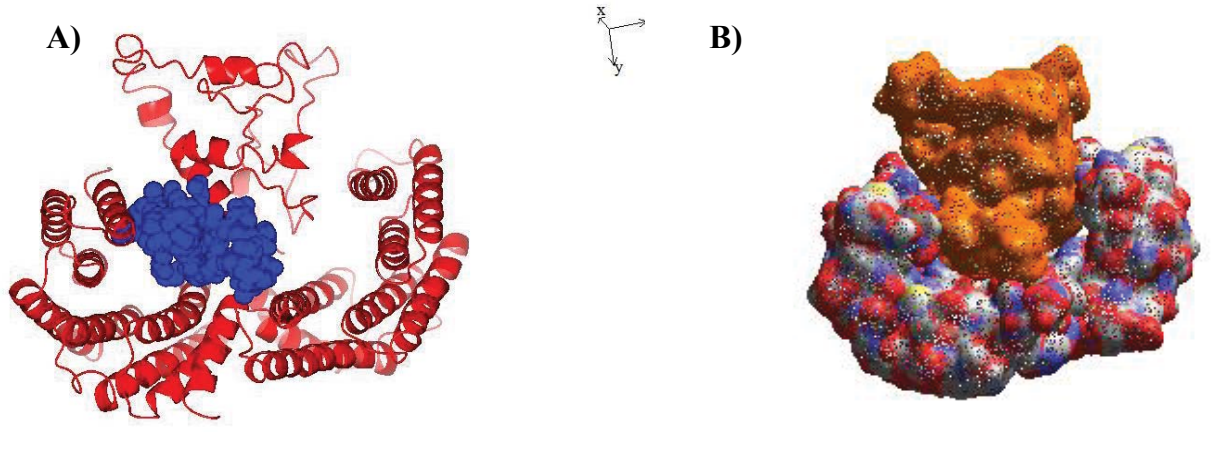

Figure 4: A: Ribbon structure (red) of S186F-SARS-COV-2 Nucleocapsid protein (NCp) and protein 14-3-3 complex, rendered as described in method section. Blue spheres show the phosphorylation rich domain,
B: Realistic rendering of S186F SARS-COV-2 Nucleocapsid protein (NCp (yellow) and protein 14-3-3 (red and blue) complex.

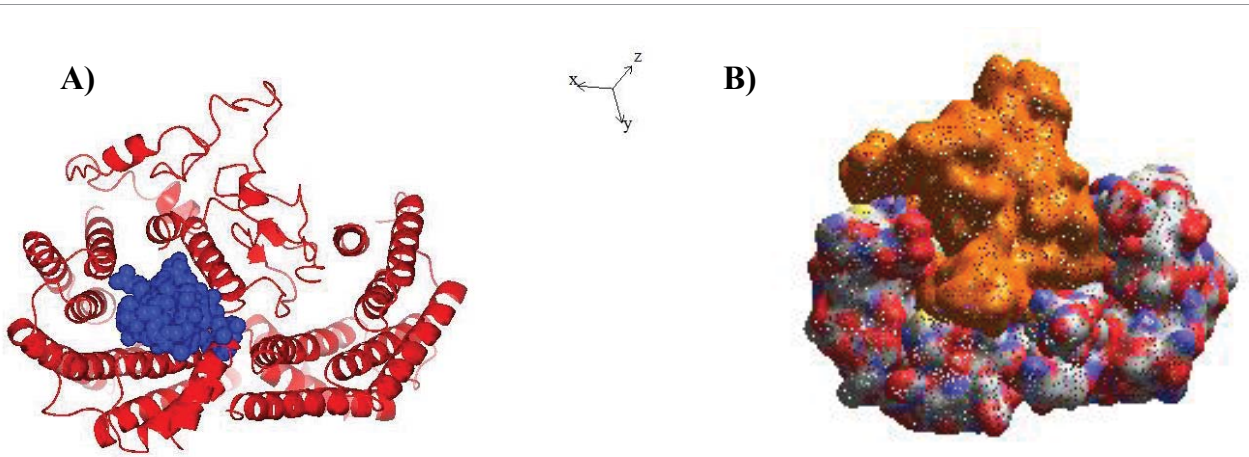

Figure 5: A: Ribbon structure (red) of phospho-serine 197-SARS-COV-2 Nucleocapsid protein (NCp) and protein 14-3-3 complex, rendered as described in method section. Phosphorylation rich domain is shown as blue spheres.
B: Realistic rendering of phospho-serine 186-SARS-COV-2 Nucleocapsid protein (NCp) (yellow) and protein 14-3-3 pre-complex (Red and Blue).

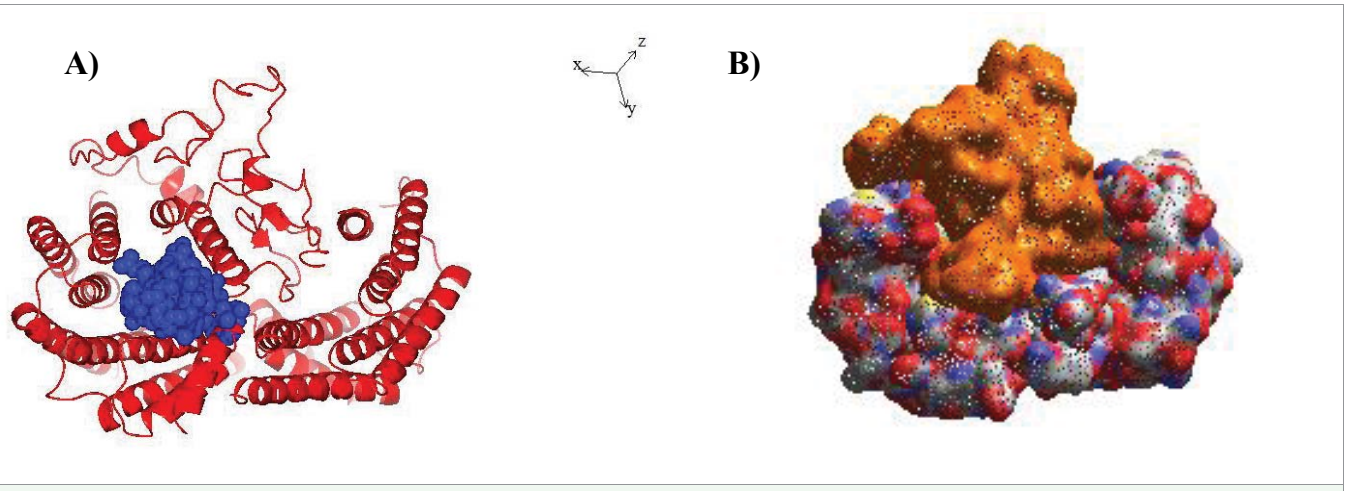

Figure 6: A: Ribbon structure (red) of S186F-SARS-COV-2 Nucleocapsid protein (NCp) and protein 14-3-3 complex, rendered as described in Method Section. Blue spheres show the phosphorylation rich domain,
B: Realistic rendering of S186F SARS-COV-2 Nucleocapsid protein (NCp (yellow) and protein 14-3-3 (red and blue) complex.

Table 1: Thermodynamic calculations, including Stability (ΔGstability), Binding Energy (ΔΔGbinding energy) and Binding Energy Difference (ΔΔΔGbinding energy difference) that underlie the sequestration of various mutant-NCps by Protein 14-3-3.

	Stability Energy (ΔG) Kcal/mol	Binding Energy (ΔΔG) Kcal/mol	Binding Energy Difference (ΔΔΔG) Kcal/mol
-Phospho-serine 186-NCp-14-3-3 complex	~605	~215	~ 0000
-S186F mutant-NCp-14-3-3 complex	~597	~208	~ -7
-Phospho-serine 186-S197L complex	~471	~81	~ -134
mutant-NCp-14-3-3 complex			
-Phospho-serine 186-S202N	~602	~213	~ -2
mutant-NCp-14-3-3 complex			
-Phospho-serine 186-RG203/204KR	~472	~39	~ -176
mutant-NCp-14-3-3 complex			
-Phospho-serine 186-RG203/204KT	~549	~164	~ -51
mutant-NCp-14-3-3 complex			
-Phospho-serine 197-NCp-14-3-3 complex	**~639**	**~244**	**~ 0000**
-S197L mutant-NCp-14-3-3 complex	~559	~170	~ -74
-Phospho-serine 197-S186F	~597	~204	~ -40
mutant-NCp-14-3-3 complex			
-Phospho-serine 197-S202N	~579	~183	~ -61
mutant-NCp-14-3-3 complex			
-Phospho-serine 197-RG203/204KR	~537	~146	~ -98
mutant-NCp-14-3-3 complex			
-Phospho-serine 197-RG203/204KT	~571	~181	~ -63
mutant-NCp-14-3-3 complex			
-Phospho-serine 202-NCp-14-3-3 complex	**~574**	**~184**	**~ 0000**
-S202N-mutant-NCp-14-3-3 complex	~499	~112	~ -72
-Phospho-serine 202-S186F	~591	~125	~ -59
mutant-NCp-14-3-3 complex			
-Phospho-serine 202-S197L	~464	~80	~ -104
mutant-NCp-14-3-3 complex			
-Phospho-serine 202-RG203/204KR	~540	~151	~ -33
mutant-NCp-14-3-3 complex			
-Phospho-serine 202-RG203/204KT	~568	~129	~ -55
mutant-NCp-14-3-3 complex			

NCp mutant-Protein 14-3-3 complexes. The results show that mutations of serines 186, 197 and 202, and arginine 203 and glycine 204 were accompanied by decreases in Stability Energy ($\Delta G_{stability\ energy}$) and Binding Energy ($\Delta\Delta G_{binding\ energy}$) indicating that there were enhanced instability of the NCp-14-3-3 complex and lowering of binding affinity between NCp and Protein 14-3-3. Calculation of the Binding Energy Difference ($\Delta\Delta\Delta G_{binding\ energy\ difference}$) between the wild type and the mutated complexes confirmed these conclusions.

DISCUSSION

Several reports have documented mutations in SARS-COV-2 Nucleocapsid protein (NCp). However, their significance remains to be fully characterized [30-36]. Because of mutations that occur in NCp, Wang et al. [34] have described it as "the worst target of any drug, vaccine, and diagnostic development". Rahmani et al. [31] appear to agree with Wang et al. [34] as they showed that the double mutation, RG203/204RK destabilized and decreased structural flexibility of NCp. However, the effects of mutations in NCp on its function were only speculated upon. On the other hand, Dutta et al. [37] have proposed that NCp could be an ideal target for vaccine development because they suggested that NCp was mutating at a relatively low rate. The roles of mutations within NCp must be explored and taken into account in any drug or vaccine development program that targets NCp. Furthermore, in view of the fact that multi-sites phosphorylation of NCp affects its function significantly [13-18], any drug or vaccine development program that targets NCp cannot ignore the fact that phosphorylations of NCp can prevent its binding to chemical ligands or antibodies.

It was previously shown that SARS-COV-2 Nucleocapsid protein (NCp) contains a phosphorylation rich domain which forms phosphorylation dependent binding and sequestration sites for Protein 14-3-3 [13,18]. The main phosphorylation dependent and sequestration sites for Protein 14-3-3 consist of the motifs, RNpSTP and RGTpSP (phospho-serines 197 and 206) which are recognized and phosphorylated by C-TAK1, a protein kinase which plays a key role in the control of the cell cycle, [13,18,38-41]. Apart from the sites that are recognized and phosphorylated by C-TAK1, there are other phosphorylation sites, including serine 186, threonine 198, serine 202 and serine 205 [13-18]. Mutations in the phosphorylation sites, serine 186, 197 and 202, and arginine 203 and glycine 204 were identified in strains/substrains isolated from individuals located in various parts of the world. It was proposed that mutations in the phosphorylation sites and phosphorylation recognition motifs allow NCp to evade sequestration by Protein 14-3-3 which would result in enhanced dimerization of NCp, an important step for NCp to act as an essential co-factor for the replication, transcription and packaging of the SARS-COV-2 genome [13,18].

In the present work, through structure model analysis and thermodynamic calculation, we have determined the effects of the above described mutations on the binding and sequestration of NCp by Protein 14-3-3. Our results show that mutations within the phosphorylation domain of NCp, including S186F, S197L, S202N, RG203/204KR and RG203/204KT were accompanied by destabilization and decreased binding efficiency of the components of the NCp mutants-Protein 14-3-3 complexes. The present work provides direct evidence that mutations in NCp affects its dimerization which determines its function as an essential co-factor of replication, transcription and packaging of the SARS-COV-2 genome [1-17].

It is submitted that mutations of the phosphorylation sites, S186F, S197L and S202N and phosphorylation recognition sites, S197L within the phosphorylation recognition motif RNpSTP and, RG203/204KR and RG203/204KT within the phosphorylation motifs, RGTpSP is a mechanism that allows NCp to escape sequestration by 14-3-3 and inhibition of its dimerization. Molecules that act to enhance the sequestration of NCp by Protein 14-3-3 and prevent the dimerization of NCp are potential therapeutics for the control of SARS-COV-2 infection and virulence. Because dimerization of NCp is an essential step for it to act as a co-factor for the replication, transcription and packaging of the SARS-COV-2 genome [1-17], molecules that act to enhance the sequestration of NCp by Protein 14-3-3 and prevent the dimerization of NCp would in principle shut off the replication, transcription and packaging of the SARS-COV-2 genome. One chemical molecule that act to prevent the dimerization of NCp at picomolar concentration has been identified and is being characterized (Limtung, P. and Tung, H.Y.L., manuscript in preparation).

ACKNOWLEDGEMENTS

This work was supported by the Nacbraht Biomedical Research Institute Fund.

AUTHOR CONTRIBUTIONS

H.Y. Lim Tung came up with the concept and the questions, performed the experiments with Pierre Limtung, analyzed the results with Pierre Limtung and wrote the paper with Pierre Limtung.

Pierre Limtung performed the experiments with H.Y. Lim Tung, analyzed the results with H.Y. Lim Tung and wrote the paper with Pierre Limtung.

REFERENCES

1. Stertz S, Reichelt M, Spiegel M, Kuri T, Martínez-Sobrido L, García-Sastre A, Weber F, Kochs G. The intracellular sites of early replication and budding of SARS-coronavirus. Virology. 2007 May 10;361(2):304-15. doi: 10.1016/j.virol.2006.11.027. Epub 2007 Jan 8. PMID: 17210170; PMCID: PMC7103305.

2. Verheije MH, Hagemeijer MC, Ulasli M, Reggiori F, Rottier PJ, Masters PS, de Haan CA. The coronavirus nucleocapsid protein is dynamically associated with the replication-transcription complexes. J Virol. 2010 Nov;84(21):11575-9. doi: 10.1128/JVI.00569-10. Epub 2010 Aug 25. PMID: 20739524; PMCID: PMC2953146.

3. Cong Y, Ulasli M, Schepers H, Mauthe M, V'kovski P, Kriegenburg F, Thiel V, de Haan CAM, Reggiori F. Nucleocapsid Protein Recruitment to Replication-Transcription Complexes Plays a Crucial Role in Coronaviral Life Cycle. J Virol. 2020 Jan 31;94(4):e01925-19. doi: 10.1128/JVI.01925-19. PMID: 31776274; PMCID: PMC6997762.

4. Tylor S, Andonov A, Cutts T, Cao J, Grudesky E, Van Domselaar G, Li X, He R. The SR-rich motif in SARS-CoV nucleocapsid protein is important for virus replication. Can J Microbiol. 2009 Mar;55(3):254-60. doi: 10.1139/w08-139. PMID: 19370068.

5. Chen CY, Chang CK, Chang YW, Sue SC, Bai HI, Riang L, Hsiao CD, Huang TH. Structure of the SARS coronavirus nucleocapsid protein RNA-binding dimerization domain suggests a mechanism for helical packaging of viral RNA. J Mol Biol. 2007 May 11;368(4):1075-86. doi: 10.1016/j.jmb.2007.02.069. Epub 2007 Mar 2. PMID: 17379242; PMCID: PMC7094638.

6. Hsieh PK, Chang SC, Huang CC, Lee TT, Hsiao CW, Kou YH, Chen IY, Chang CK, Huang TH, Chang MF. Assembly of severe acute respiratory syndrome coronavirus RNA packaging signal into virus-like particles is nucleocapsid dependent. J Virol. 2005 Nov;79(22):13848-55. doi: 10.1128/JVI.79.22.13848-13855.2005. PMID: 16254320; PMCID: PMC1280188.

7. Yu IM, Gustafson CL, Diao J, Burgner JW 2nd, Li Z, Zhang J, Chen J.

Recombinant severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein forms a dimer through its C-terminal domain. J Biol Chem. 2005 Jun 17;280(24):23280-6. doi: 10.1074/jbc.M501015200. Epub 2005 Apr 21. PMID: 15849181.

8. He R, Dobie F, Ballantine M, Leeson A, Li Y, Bastien N, Cutts T, Andonov A, Cao J, Booth TF, Plummer FA, Tyler S, Baker L, Li X. Analysis of multimerization of the SARS coronavirus nucleocapsid protein. Biochem Biophys Res Commun. 2004 Apr 2;316(2):476-83. doi: 10.1016/j.bbrc.2004.02.074. PMID: 15020242; PMCID: PMC7111152.

9. Peng TY, Lee KR, Tarn WY. Phosphorylation of the arginine/serine dipeptide-rich motif of the severe acute respiratory syndrome coronavirus nucleocapsid protein modulates its multimerization, translation inhibitory activity and cellular localization. FEBS J. 2008 Aug;275(16):4152-63. doi: 10.1111/j.1742-4658.2008.06564.x. Epub 2008 Jul 9. PMID: 18631359; PMCID: PMC7164085.

10. Surjit M, Liu B, Kumar P, Chow VT, Lal SK. The nucleocapsid protein of the SARS coronavirus is capable of self-association through a C-terminal 209 amino acid interaction domain. Biochem Biophys Res Commun. 2004 May 14;317(4):1030-6. doi: 10.1016/j.bbrc.2004.03.154. PMID: 15094372; PMCID: PMC7111157.

11. Luo H, Ye F, Sun T, Yue L, Peng S, Chen J, Li G, Du Y, Xie Y, Yang Y, Shen J, Wang Y, Shen X, Jiang H. In vitro biochemical and thermodynamic characterization of nucleocapsid protein of SARS. Biophys Chem. 2004 Dec 1;112(1):15-25. doi: 10.1016/j.bpc.2004.06.008. PMID: 15501572; PMCID: PMC7116930.

12. Chang CK, Sue SC, Yu TH, Hsieh CM, Tsai CK, Chiang YC, Lee SJ, Hsiao HH, Wu WJ, Chang CF, Huang TH. The dimer interface of the SARS coronavirus nucleocapsid protein adapts a porcine respiratory and reproductive syndrome virus-like structure. FEBS Lett. 2005 Oct 24;579(25):5663-8. doi: 10.1016/j.febslet.2005.09.038. Epub 2005 Sep 30. PMID: 16214138; PMCID: PMC7094587.

13. Tung HYL, Limtung P. Mutations in the phosphorylation sites of SARS-CoV-2 encoded nucleocapsid protein and structure model of sequestration by protein 14-3-3. Biochem Biophys Res Commun. 2020 Oct 29;532(1):134-138. doi: 10.1016/j.bbrc.2020.08.024. Epub 2020 Aug 15. PMID: 32829876; PMCID: PMC7428706.

14. Surjit M, Kumar R, Mishra RN, Reddy MK, Chow VT, Lal SK. The severe acute respiratory syndrome coronavirus nucleocapsid protein is phosphorylated and localizes in the cytoplasm by 14-3-3-mediated translocation. J Virol. 2005 Sep;79(17):11476-86. doi: 10.1128/JVI.79.17.11476-11486.2005. PMID: 16103198; PMCID: PMC1193639.

15. Wu CH, Yeh SH, Tsay YG, Shieh YH, Kao CL, Chen YS, Wang SH, Kuo TJ, Chen DS, Chen PJ. Glycogen synthase kinase-3 regulates the phosphorylation of severe acute respiratory syndrome coronavirus nucleocapsid protein and viral replication. J Biol Chem. 2009 Feb 20;284(8):5229-39. doi: 10.1074/jbc.M805747200. Epub 2008 Dec 23. PMID: 19106108.

16. Wu CH, Chen PJ, Yeh SH. Nucleocapsid phosphorylation and RNA helicase DDX1 recruitment enables coronavirus transition from discontinuous to continuous transcription. Cell Host Microbe. 2014 Oct 8;16(4):462-72. doi: 10.1016/j.chom.2014.09.009. PMID: 25299332; PMCID: PMC7104987.

17. Carlson CR, Asfaha JB, Ghent CM, Howard CJ, Hartooni N, Morgan DO. Phosphorylation modulates liquid-liquid phase separation of the SARS-CoV-2 N protein. bioRxiv [Preprint]. 2020 Jun 29:2020.06.28.176248. doi: 10.1101/2020.06.28.176248. PMID: 32637943; PMCID: PMC7337373.

18. Tung HYL, Limtung, P. Structure Model Analysis Of Phosphorylation Dependent Binding And Sequestration Of SARS-COV-2 Encoded Nucleocapsid Protein By Protein 14-3-3. bioRxiv. 2020. doi: 10.1101/2020.09.16.299362

19. Xu D, Zhang Y. Ab initio protein structure assembly using continuous structure fragments and optimized knowledge-based force field. Proteins. 2012 Jul;80(7):1715-35. doi: 10.1002/prot.24065. Epub 2012 Apr 13. PMID: 22411565; PMCID: PMC3370074.

20. Xu D, Zhang Y. Toward optimal fragment generations for ab initio protein structure assembly. Proteins. 2013 Feb;81(2):229-39. doi: 10.1002/prot.24179. Epub 2012 Oct 16. PMID: 22972754; PMCID: PMC3551984.

21. Guerois R, Nielsen JE, Serrano L. Predicting changes in the stability of proteins and protein complexes: a study of more than 1000 mutations. J Mol Biol. 2002 Jul 5;320(2):369-87. doi: 10.1016/S0022-2836(02)00442-4. PMID: 12079393.

22. Schymkowitz J, Borg J, Stricher F, Nys R, Rousseau F, Serrano L. The FoldX web server: an online force field. Nucleic Acids Res. 2005 Jul 1;33(Web Server issue):W382-8. doi: 10.1093/nar/gki387. PMID: 15980494; PMCID: PMC1160148.

23. Pierce BG, Wiehe K, Hwang H, Kim BH, Vreven T, Weng Z. ZDOCK server: interactive docking prediction of protein-protein complexes and symmetric multimers. Bioinformatics. 2014 Jun 15;30(12):1771-3. doi: 10.1093/bioinformatics/btu097. Epub 2014 Feb 14. PMID: 24532726; PMCID: PMC4058926.

24. Benzinger A, Popowicz GM, Joy JK, Majumdar S, Holak TA, Hermeking H. The crystal structure of the non-liganded 14-3-3sigma protein: insights into determinants of isoform specific ligand binding and dimerization. Cell Res. 2005 Apr;15(4):219-27. doi: 10.1038/sj.cr.7290290. PMID: 15857576.

25. McNicholas S, Potterton E, Wilson KS, Noble ME. Presenting your structures: the CCP4mg molecular-graphics software. Acta Crystallogr D Biol Crystallogr. 2011 Apr;67(Pt 4):386-94. doi: 10.1107/S0907444911007281. Epub 2011 Mar 18. PMID: 21460457; PMCID: PMC3069754.

26. Garden DP, Zhorov BS. Docking flexible ligands in proteins with a solvent exposure- and distance-dependent dielectric function. J Comput Aided Mol Des. 2010 Feb;24(2):91-105. doi: 10.1007/s10822-009-9317-9. Epub 2010 Jan 30. PMID: 20119653.

27. Teng S, Srivastava AK, Schwartz CE, Alexov E, Wang L. Structural assessment of the effects of amino acid substitutions on protein stability and protein protein interaction. Int J Comput Biol Drug Des. 2010;3(4):334-49. doi: 10.1504/IJCBDD.2010.038396. Epub 2011 Feb 4. PMID: 21297231; PMCID: PMC3319068.

28. Nishi H, Tyagi M, Teng S, Shoemaker BA, Hashimoto K, Alexov E, Wuchty S, Panchenko AR. Cancer missense mutations alter binding properties of proteins and their interaction networks. PLoS One. 2013 Jun 14;8(6):e66273. doi: 10.1371/journal.pone.0066273. PMID: 23799087; PMCID: PMC3682950.

29. Nishi H, Hashimoto K, Panchenko AR. Phosphorylation in protein-protein binding: effect on stability and function. Structure. 2011 Dec 7;19(12):1807-15. doi: 10.1016/j.str.2011.09.021. PMID: 22153503; PMCID: PMC3240861.

30. Benvenuto D, Demir AB, Giovanetti M, Bianchi M, Angeletti S, Pascarella S, Cauda R, Ciccozzi M, Cassone A. Evidence for mutations in SARS-CoV-2 Italian isolates potentially affecting virus transmission. J Med Virol. 2020 Jun 3:10.1002/jmv.26104. doi: 10.1002/jmv.26104. Epub ahead of print. PMID: 32492183; PMCID: PMC7300971.

31. Rahmani, MS, Islam M.R, Rubayet ASM, Alam U, Islam I, Hoque M.Z, Akter S, Rahaman M, Sultana M, Hossaini MA. Evolutionary dynamics of SARS-CoV-2 nucleocapsid protein (N protein) and its consequences. bioRxiv. 2020. doi: 10.1101/2020.08.05.237339.

32. Zeng W, Liu G, Ma H, Zhao D, Yang Y, Liu M, Mohammed A, Zhao C, Yang Y, Xie J, Ding C, Ma X, Weng J, Gao Y, He H, Jin T. Biochemical characterization of SARS-CoV-2 nucleocapsid protein. Biochem Biophys Res Commun. 2020 Jun 30;527(3):618-623. doi: 10.1016/j.bbrc.2020.04.136. Epub 2020 Apr 30. PMID: 32416961; PMCID: PMC7190499.

33. Dawood AA. Mutated COVID-19 may foretell a great risk for mankind in the future. New Microbes New Infect. 2020 Apr 4;35:100673. doi: 10.1016/j.nmni.2020.100673. PMID: 32292587; PMCID: PMC7129032.

34. Wang R, Hozumi Y, Yin C, Wei GW. Decoding SARS-CoV-2 Transmission and Evolution and Ramifications for COVID-19 Diagnosis, Vaccine, and Medicine. J Chem Inf Model. 2020 Jun 25:acs.jcim.0c00501. doi: 10.1021/acs.jcim.0c00501. Epub ahead of print. PMID: 32530284; PMCID: PMC7318555.

35. Guruprasad K. Amino acid mutations in the protein sequences of human SARS CoV-2 Indian isolates compared to Wuhan-Hu-1 reference isolate from China. chemRxiv. 2020. doi: 10.26434/chemrxiv.12300860.v1

36. Maitra, A., Sarkar, M. C., Raheja, H., Biswas, N. K., Chakraborti, S., Singh, A. K., Ghosh, S., Sarkar, S., Patra, S., Mondal, R. K., Ghosh, T., Chatterjee, A., Maitra A, Sarkar MC, Raheja H, Biswas NK, Chakraborti S, Singh AK, Ghosh S, Sarkar S, Patra S, Mondal RK, Ghosh T, Chatterjee A, Banu H, Majumdar A, Chinnaswamy S, Srinivasan N, Dutta S, DAS S. Mutations in SARS-CoV-2 viral RNA identified in Eastern India: Possible implications for the ongoing outbreak in India and impact on viral structure and host susceptibility. J Biosci. 2020;45(1):76. doi: 10.1007/s12038-020-00046-1. PMID: 32515358; PMCID: PMC7269891.

37. Dutta NK, Mazumdar K, Gordy JT. The Nucleocapsid Protein of SARS-CoV-2: a Target for Vaccine Development. J Virol. 2020 Jun 16;94(13):e00647-20. doi: 10.1128/JVI.00647-20. PMID: 32546606; PMCID: PMC7307180.

38. Ogg S, Gabrielli B, Piwnica-Worms H. Purification of a serine kinase that associates with and phosphorylates human Cdc25C on serine 216. J Biol Chem. 1994 Dec 2;269(48):30461-9. PMID: 7982962.

39. Müller J, Ritt DA, Copeland TD, Morrison DK. Functional analysis of C-TAK1 substrate binding and identification of PKP2 as a new C-TAK1 substrate. EMBO J. 2003 Sep 1;22(17):4431-42. doi: 10.1093/emboj/cdg426. PMID: 12941695; PMCID: PMC202368.

40. Muslin AJ, Tanner JW, Allen PM, Shaw AS. Interaction of 14-3-3 with signaling proteins is mediated by the recognition of phosphoserine. Cell. 1996 Mar 22;84(6):889-97. doi: 10.1016/s0092-8674(00)81067-3. PMID: 8601312.

41. Yaffe MB, Rittinger K, Volinia S, Caron PR, Aitken A, Leffers H, Gamblin SJ, Smerdon SJ, Cantley LC. The structural basis for 14-3-3:phosphopeptide binding specificity. Cell. 1997 Dec 26;91(7):961-71. doi: 10.1016/s0092-8674(00)80487-0. PMID: 9428519.