Vitamin B12 may inhibit RNA-dependent-RNA polymerase activity of nsp12 from the SARS-CoV-2 Virus

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Abstract

SARS-CoV-2 is the causative agent for the ongoing COVID19 pandemic, and this virus belongs to the Coronaviridae family. Like other members of this family, the virus possesses a positive-sense single-stranded RNA genome. The genome encodes for the nsp12 protein, which houses the RNA-dependent-RNA polymerase (RdRP) activity responsible for the replication of the viral genome. A homology model of nsp12 was prepared using the structure of the SARS nsp12 (6NUR) as a model. The model was used to carry out *in silico* screening to identify molecules among natural products, or FDA approved drugs that can potentially inhibit the activity of nsp12. This exercise showed that vitamin B12 (methylcobalamin) may bind to the active site of the nsp12 protein. A model of the nsp12 in complex with substrate RNA and incoming NTP showed that Vitamin B12 binding site overlaps with that of the incoming nucleotide. A comparison of the calculated energies of binding for RNA plus NTP and methylcobalamin suggested that the vitamin may bind to the active site of nsp12 with significant affinity. It is, therefore, possible that methylcobalamin binding may prevent association with RNA and NTP and thus inhibit the RdRP activity of nsp12. Overall, our computational studies suggest that methylcobalamin form of vitamin B12 may serve as an effective inhibitor of the nsp12 protein.

Introduction

The members of the Coronaviridae family are viruses with positive-sense single-stranded RNA genomes (1). At present, some members of this family, such as SARS, MERS, and SARS-CoV-2 represent pathogens of great concern to public health. SARS-CoV-2 is responsible for the ongoing pandemic of COVID-19, which started in the city of Wuhan in China, has now spread to more than 150 countries. At present, there are nearly 700000 confirmed cases of the disease caused by this pathogen, with more than 30000 fatalities (2). At present, there are no effective treatments available against COVID-19, and current medical protocols involve isolating the patient and provide symptomatic treatment for patients with mild disease and oxygen therapy/ventilator support for patients with severe disease.

The genome of COVID-19 is roughly 30 kB long with a gene at the 5' end known as *orf1ab* that encodes for all the polyprotein bearing all the non-structural proteins (3). The virus also possesses genes that code for structural proteins, namely spike (S), envelope (E), membrane (M) and nucleocapsid (N) (4). The polyprotein arising from *orf1ab* may undergo proteolytic processing to give rise to 16 proteins namely nsPs 1-16 (5). Among the cleaved products of the ORF1Ab polyprotein, the proteins of known function include nsp3 which has an adenosine diphosphate-ribose 1"-phosphatase activity (6). The protease activity that is responsible for the cleavage of the polyprotein is present in the nsp5 protein. The nsp12 protein houses the RNA-dependent RNA polymerases that is responsible for duplication of the genome. The RNA helicase activity that is critical for genome duplication is present in the nsp13 protein. Exoribonuclease (exoN) and N7-methyltransferase activities are present in the nsp14 protein (7). The nsp15 protein houses a Nidoviral ribonuclease specific for U, and the nsp16 protein has a SAM-dependent O-methyltransferase activity (3).

There is an urgent need for the identification of new molecules that can reduce viral titers and thus limit the severity of the disease. Towards this end, we have generated a model of the nsp12 molecule from SARS-CoV-2 (SCV2-nsp12) and used this model to carry out *in silico* screening to identify potential inhibitors of the RNA-

dependent-RNA polymerase activity. Our studies suggest that the methylcobalamin form of Vitamin B12 may serve as an effective inhibitor of the RdRp activity of nsp12.

Methods

Homology Modelling of nsp12 and Model of the functional ternary complex

The sequence corresponding to nsp12 from a sequence of SARS-CoV-2 deposited in Genbank by the CDC (Atlanta, USA) with the accession no. MT044257.1 was used. The nsp12 protein from SARS-CoV exhibits 97% identity with SCV2-nsp12. A homology model was generated using the SWISSMOD server (8), and the structure of nsp12 from the nsp12-nsp7-nsp8 complex (6NUR) ofSARS-CoV was used as a template (9). The validation of the model was carried out by the SWISS MOD server (8).

To generate a computational model of the functional ternary complex (SCV2-nsp12:RNA:NTP), initially DALI searches were carried with the model of apo- structure to identify structural orthologues of nsp12 bound to RNA and incoming nucleotide (10). These searches showed that, on superimposition, the Q-beta replicase exhibits significant structural homology in the palm domain. The RNA and incoming nucleotide of the transformed coordinates of the ternary complex of Q-beta replicase (3AVX) were transferred onto the homology model of SCV2-nsp12 (11). The local structure in the active site was altered manually to ensure co-ordination of the co-factor ion by the catalytic residues and the triphosphate moiety of the incoming nucleotide as seen previously for DNA polymerases (12). The structure prepared in this way was subjected to energy minimization using DESMOND module of the Schrödinger suite. The structure was minimized in an orthorhombic box containing single point and charge water model and subjected to steepest descent and LBGFS vectors minimization until the difference in energy converged to 0.1 kcal/mol (13).

In Silico Screening

The model of SCV2-nsp12 was imported into MAESTRO interface of SCHRODINGER suite and prepared using protein preparation wizard program. The prepared structure was used to identify potential binding sites using the SITEMAP program, which uses site score function to rank the possible binding sites according to

size, functionality, and extent of solvent exposure on the protein (14). Sites with a site score value of ≥0.8 were identified and further examined using PyMOL to see if it could inhibit the RNA binding. Residues spanning the most relevant site were used to prepare a receptor grid using the Receptor Grid Generation module in Schrödinger.

Concurrently various annotated libraries FDA approved drugs, natural products, antiviral compounds and drug repurposing compounds were downloaded from selleckchem.com in the SDF format (15). The downloaded files were converted to accurate energy minimized 3D molecular structures using LIGPREP module in SCHRODINGER. LIGPREP was used to expand tautomeric states, ionization states, ring conformations, and stereoisomers of ligand molecules to produce broad chemical and structural diversity from each molecule along with the correction of Lewis structures and ligand order of these molecules.

Using the glide docking module the prepared grid for the binding site in protein and the library of prepared ligands from LIGPREP were docked. GLIDE generates multiple poses for the ligands, which are initially filtered by spatial fit onto proteins active site and are checked for the complementarity of interaction using ChemScore function. The poses that pass the initial filter are minimized with respect to the receptor grid using OPLS-AA non-bonded ligand-receptor interaction energy. Once the energy is calculated GLIDEScore multi-ligand scoring function assigned scores to the poses. GLIDE docking was carried out in standard-precision (SP) mode, and the molecules that bind to the receptors with good docking score and negative binding energy were used for further analysis.

Minimization of the ternary complex of nsp12 and the nsp12-methylcobalamin complex.

The SCV2-nsp12: methylcobalamin complex were then subjected to energy minimization using the DESMOND module of SCHRODINGER suite. The system setup program was used to set up an orthorhombic boundary box containing a simple point-charge water model and 11 Na^+ ions to neutralize the system. This system was then minimized using steepest descent and LBGFS vectors with a convergence threshold of 0.1 kcal/mol. The minimized models of the ternary complex of nsp12 (SCV2-

nsp12:RNA:GTP:2 Ca^{2+} ions) and the nsp12:methylcobalamin complex were analyzed using CONTACT program in CCP4 suite to identify the residues involved in interactions with the substrates and the vitamin, respectively (16). The models of the complexes were superimposed using the COOT program (17) and all figures were prepared using PYMOL (Schrödinger Inc.)

Comparison of binding energies

The binding energy of SCV2-nsp12 with substrate or methylcobalamin were calculated by Molecular Mechanics energies combined with Generalized Born and Surface Area continuum solvation (MMGBSA) program in the Schrödinger suite. The docked protein and ligand complex were separated manually and loaded as receptor or ligand, respectively in the MMGBSA module. MMGBSA program minimizes each of them separately as well as in combination using VSGB 2.0 (Variable dielectric Surface Generalized Born) solvation model. The energy of binding is then calculated by subtracting the energy of the optimized free receptor and optimized free ligand from the energy of the optimized complex. Further, to check if methylcobalamin could compete with the natural substrates, the binding energy between the modelled nsp12 and RNA plus incoming nucleotide in the model of the ternary complex was also calculated.

Results

Model of the SCV2-nsp12 in its apo- and functional state

The SCV2-nsp12 protein shows 97% identity with the corresponding protein from SARS (Figure 1). The structure of the SARS-nsp12 in complex with nsp7 and nsp8 was determined using Cryo-EM and deposited in the PDB with the accession code 6NUR. The structure of nsp12 from 6NUR was used to generate a computational model of the SCV2-nsp12 in its apo- state using the SWISSMOD server (Figure 2). The server showed that the stereochemistry of the model was good, with 98% residues in the allowed regions and less than 1% residues in the disallowed regions. Swissmod uses a QMEAN (qualitative model energy analysis) to evaluate the models and the score obtained for the model was -0.65, which is good for further analysis. The model encompasses residues 4509-5311 of the polyprotein translated from *orf1ab* of the

COVID-19 genome and also includes two Zn^{2+} ions. The model shows the presence of the N-terminal extension (119-397), fingers (398-581 and 628-687), palm (582-627 and 688-815), and thumb (816-919) domains (Figure 2).

To generate the structure of the SCV2-nsp12 structure in its functional state, initially, a DALI search was carried out to identify structural orthologs of this enzyme. The list of enzymes that showed good superimposition with the nsp12 model was analyzed to identify structures of functional ternary complexes. The structure of Q-beta replicase in complex with RNA and incoming nucleotide (3AVX) gave a Z-score of 3.7 and an rmsd of 3.0 in the DALI search. The superimposition was used to generate a model of the SCV2-nsp12 protein in complex with RNA bearing template C, incoming nucleotide (dGTP) and two Ca^{2+} ions. The model was subjected to energy minimization and converged to a minimum energy of -3.97×10^5 kcal/mol. The Ramachandran plot of the minimized model showed that only 1.75% residues were in the disallowed regions. The model showed the presence of the expected octahedral co-ordination of one of the cofactor ion between the triphosphate moiety and the catalytic residues D762 and D620 (Supplementary Figure 1).

In Silico Screening identifies Vitamin B12 as a potential binder

The site map program showed four possible binding sites in the SCV2-nsp12 protein, which were ranked according to their ability to bind to various ligands. Site 2 with a site score of 0.99 was selected over site 1 with a site score of 1.03 as it overlapped with the RNA binding groove and catalytic site of the enzyme. The residues spanning site2 were used to generate a receptor grid used for molecular docking. Molecular docking was carried out using annotated libraries of molecules that could bind to SCV2-nsp12 open complex using glide dock program. The top hits from each of these libraries were ranked according to their docking score, glide gscore and glide energy based on the interaction between the protein and ligand. Vitamin B12 showed a significant docking score (-8.193), and since it is already part of many drug formulations, the vitamin molecule was selected for further analysis. It was observed that the docked B12 molecule was a Cobalt free version of the vitamin.

Consequently, the methylcobalamin ligand from the complex with human MMACHC (3SC0) was manually docked at the appropriate site in the nsp12 enzyme using the complex model output by GLIDE as a guide (18, 19). The SCV2-nsp12:methylcobalamin complex model prepared this way was then subjected to energy minimization. The SCV2-nsp12:methylcobalamin converged to a minimum energy of -4X10^5 kcal/mol. The final model of the complex shows the presence of one molecule of methylcobalamin bound in the active site of the nsp12 enzyme (Figure 3).

Comparison of interactions in SCV2-nsp12:Vitamin B12 complex and the functional ternary complex of SCV2-nsp12

The model of the ternary complex of SCV2-nsp12 was analyzed to identify the interacting residues. The residues that form Van der Wall interactions with RNA are Lys413, Asn498, Asn499, Lys502, Ser503, Lys513, Lys547, Tyr548, Val559, Arg571, Lys579, Ala582, Arg585, Ile591, Gly592, Thr593, Ser594, Lys595, Phe596, Tyr597, Ser684, Gly685, Asp686, Ala687, Ala690, Tyr691, Leu760, Ser761, Asp762, Asp763, Glu813, Cys815, Ser816, Gln817, Pro834, Asp835, Arg838, Ile839, Val850, Leu856, Glu859, Arg860, Val862, Ser863, Leu864, Ile866, Asp867, Asn913, Arg916, and Tyr917. The residues Asn498, Asn499, Lys502, Ser503, Lys513, Arg571, Lys579, Ala582, Arg585, Thr593, Ser594, Lys595, Tyr597, Cys815, Ser816, Asn913, Arg916, and Tyr917 form polar interactions with RNA. In the ternary complex, the residues Lys574, Asp620, Tyr621, Pro622, Lys623, Asp625, Ser684, Asn693, and Asp762 form polar interactions with the incoming nucleotide i. e. GTP. A624 and G685 form Van der Waal interactions with GTP. The residues Asp620, Tyr621, Asp762, Asp763, and Glu813 also interact with the incoming nucleotide through the co-factor ions. Asp762, Asp763, and Asp620 are the catalytic residues that are responsible for nucleic acid synthesis reaction.

The SCV2-nsp12:methylcobalamin complex was analyzed to identify the residues that interact with the vitamin (Figure 4). The nsp12 residues that form Van der Waal contacts with the methylcobalamin molecule include Val168, His441, Tyr457, Tyr460, Ile550, Ser551, Ala552, Lys553, Arg555, Arg557, Thr558, Lys623, Asp625, Arg626, Ser761, Asp762, Glu813, Cys815, Ser816, and Arg838. The residues Lys553,

Arg555, Thr558, Lys623, Asp625, Arg626, Asp762, Glu813, and Arg838 also form polar interactions with the vitamin B12 molecule. One of the three catalytic residues (Asp762) forms polar interactions with the methylcobalamin molecule. A superimposition of the model of the ternary complex and that of the SCV2-nsp12:methylcobalamin complex was carried out to ascertain the level of overlap between the binding sites of the natural substrates of nsp12 and the methylcobalamin molecule. The superimposition showed that the binding site of methylcobalamin overlaps with that of the incoming nucleotide and the terminal primer nucleotide of the RNA substrate (Figure 5).

Energy of binding of SCV2-nsp12 with natural substrates versus methylcobalamin

Using the MMGBSA program, the energy of interaction between SCV2-nsp12 and RNA+GTP was compared with that of between SCV2-nsp12 and methylcobalamin. The calculated binding energy for the interaction of RNA and incoming nucleotide with SCV2-nsp12 is -95 kcal/mol. In comparison, the calculated binding energy value for the interaction of methylcobalamin with SCV2-nsp12 is -48 kcal/mol. These values suggest that Vitamin B12 can bind with significant affinity at the active site of the SCV2-nsp12 enzyme. Therefore, the binding of methylcobalamin may inhibit the formation of functional ternary complex of nsp12 and thus prevent RNA synthesis necessary for replication of the viral genome.

Discussion

The studies presented here suggest that methylcobalamin may be a possible inhibitor of the RNA-dependent-RNA polymerase activity of the SCV2-nsp12 enzyme. Since this enzyme is critical for the replication of the viral enzyme, the inhibition of this enzyme can result in lower viral titres and reduce the severity of the COVID-19 disease. The number of patients suffering from COVID-19 are increasing daily and about 7% of them are in serious condition. Hence, the ability of methylcobalamin to inhibit viral replication should be tested urgently using *in vitro* and *in vivo* assays. In addition, given the urgency of the situation and the fact that methylcobalamin is already part of drug

formulations, doctors may consider adding or increasing the dosage of methylcobalamin in their current patient care protocols.

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Author contribution

DTN and NN conceived the project. NN carried out the computations and DTN supervised the work. DTN and NN analyzed the results and wrote the manuscript.

Competing Interests statement: None declared

Figure Legends:

Figure 1: Alignment of the sequence of SCV2-nsp12 (119-901) with the sequence of the available structure of SARS-nsp12 (6NUR). The Covid19-nsp12 sequence exhibits about 97% identity with the corresponding stretch in SARS-nsp12.

Figure 2: Model of the SCV2-nsp12 enzyme in its apo- state. The N-terminal extension region and the palm, fingers and thumb domains are shown in green, cyan, yellow and orange, respectively. The catalytic residues Asp620, Asp762 and Asp763 are coloured according to element and shown in stick representation.

Figure 3: Model of the SCV2-nsp12 enzyme in complex with methylcobalamin. The Vitamin B12 molecule is shown in stick representation and coloured according to element. The N-terminal extension region and the palm, fingers and thumb domains are shown in green, cyan, yellow and orange, respectively. The catalytic residues Asp620, Asp762 and Asp763 are coloured according to element and shown in stick representation.

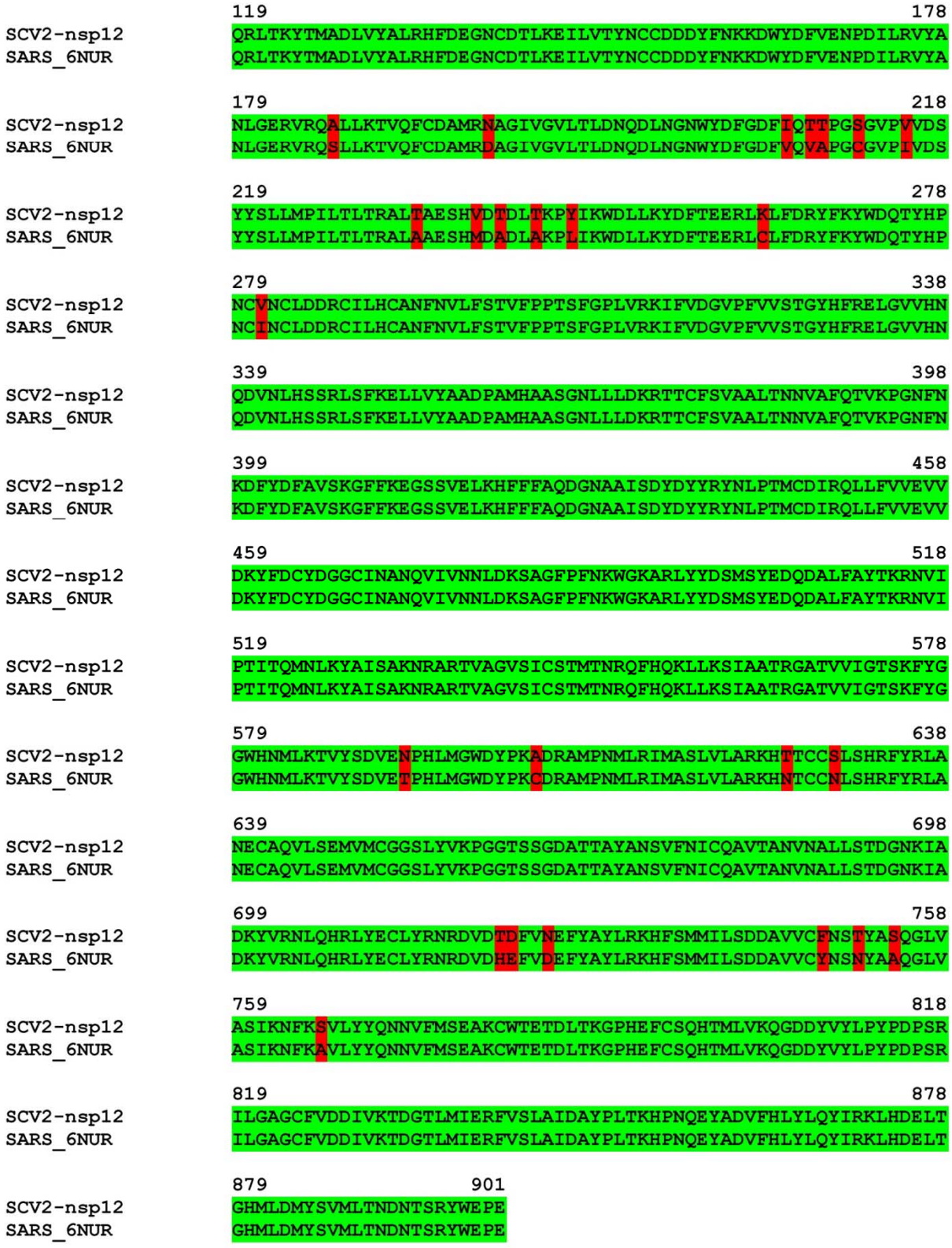
Figure 4: Residues of SCV2-nsp12 that interact with methylcobalamin. The Vitamin B12 molecule is shown in stick representation and coloured according to element. The N-terminal extension region and the palm, fingers and thumb domains are shown in green, cyan, yellow and orange, respectively. The interacting residues are coloured according to element and shown in stick representation.

Figure 5: Binding site of methylcobalamin overlaps with that of incoming nucleotide. (A) Superimposition of the models of the functional ternary complex (cyan) and that of SCV2-nsp12:methylcobalamin (green) is displayed. The protein chains are shown in ribbon representation. The catalytic residues, incoming GTP and methylcobalamin are shown in stick representation and the cofactor ions are shown as spheres (B) The

surface of the protein molecule is displayed and the RNA, incoming nucleotide, and methylcobalamin are shown in stick representation and coloured white, red and magenta, respectively. The vitamin B12 binding site overlaps with that of the incoming nucleotide and the terminal primer nucleotide of the RNA substrate.

Supplementary Figure Legends:

Supplementary Figure 1: Model of the functional ternary complex of the SCV2-nsp12 enzyme in complex. The RNA substrate and incoming GTP are shown in stick representation and coloured according to element. The N-terminal extension region and the palm, fingers and thumb domains are shown in green, cyan, yellow and orange, respectively. The catalytic residues Asp620, Asp762 and Asp763 are coloured according to element and shown in stick representation and the cofactor ions are shown as blue spheres.



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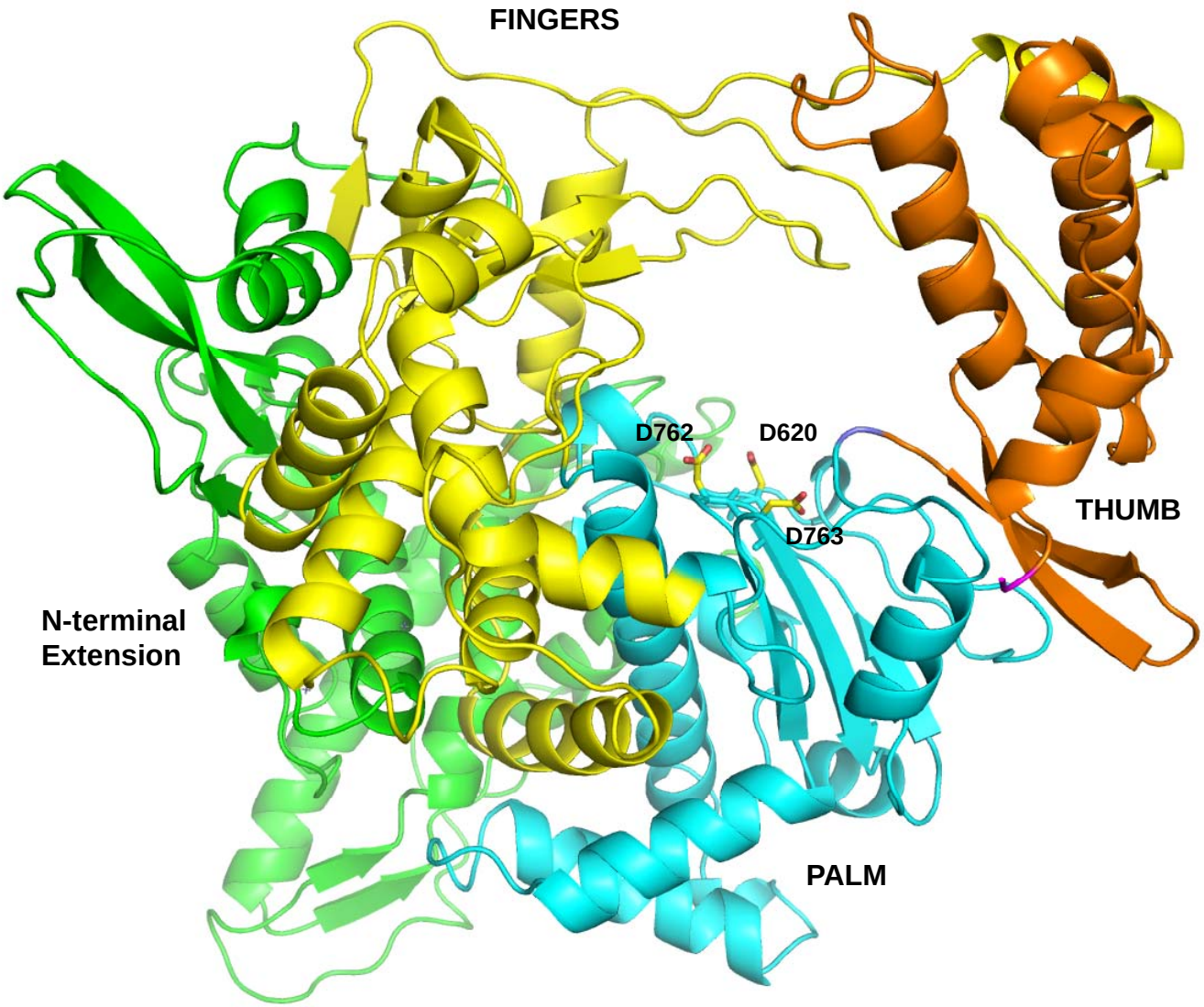

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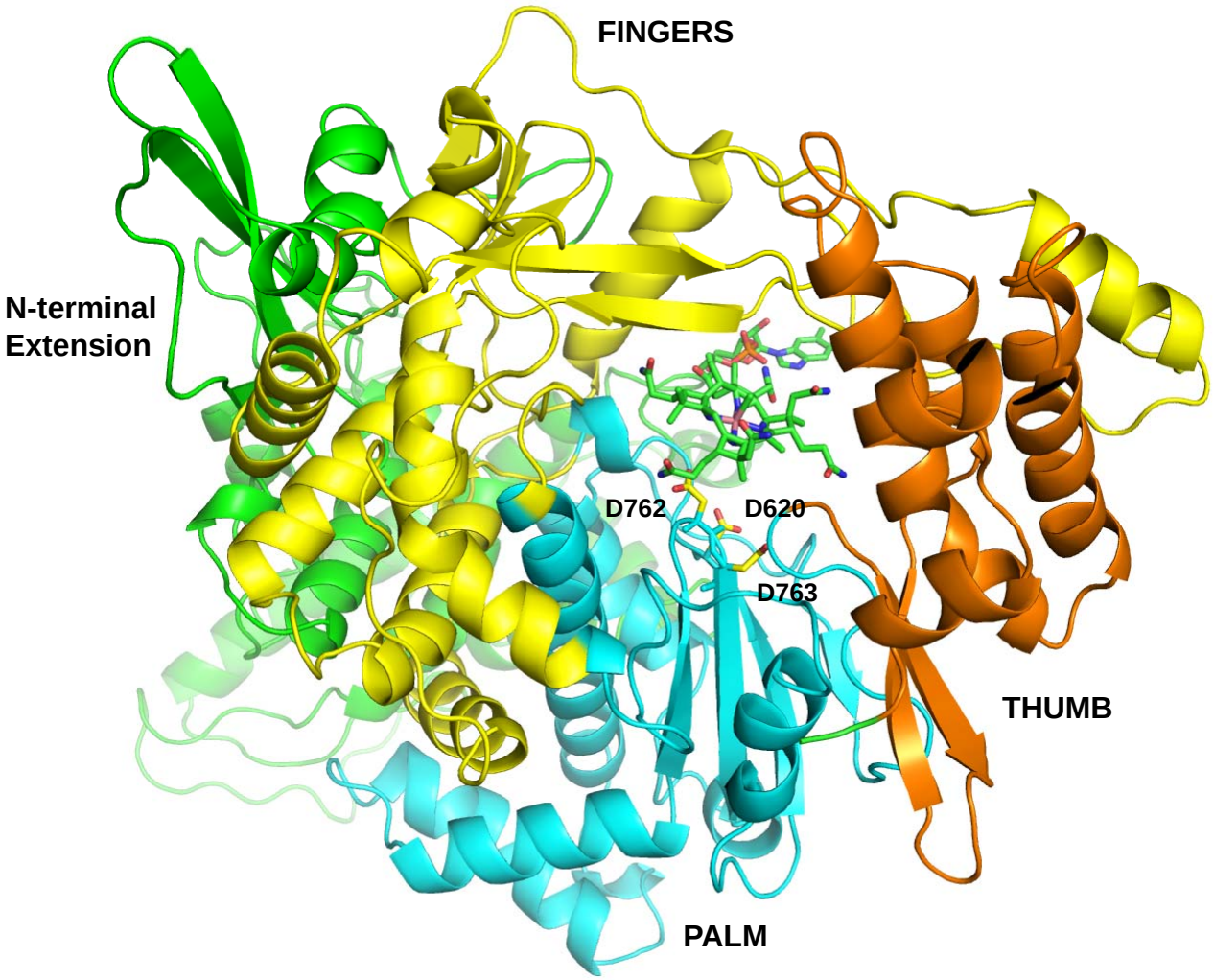

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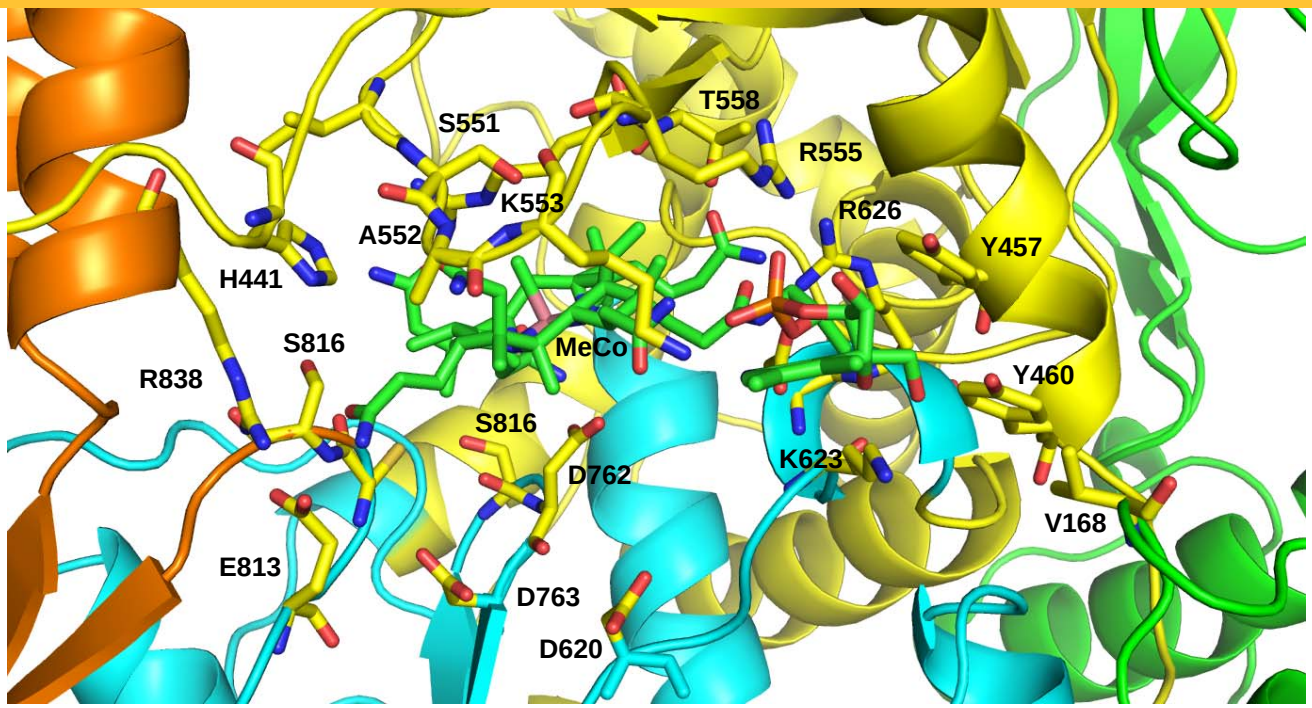
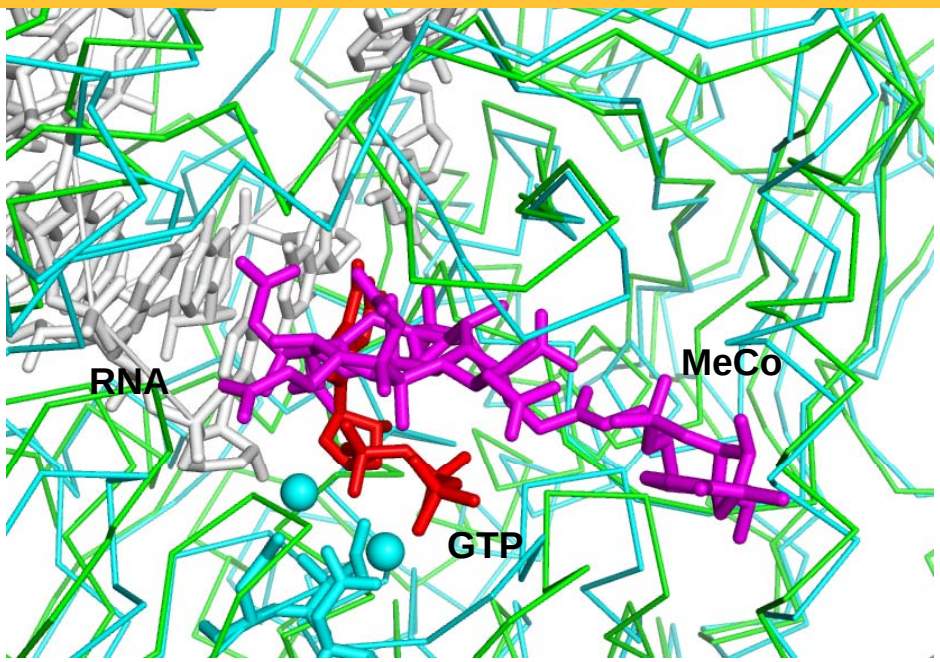


Figure 4: Residues of SCV2-nsp12 that interact with methylcobalamin. The Vitamin B12 (MeCo) molecule is shown in stick representation and coloured according to element. The N-terminal extension region and the palm, fingers and thumb domains are shown in green, cyan, yellow and orange, respectively. The interacting residues are coloured according to element and shown in stick representation.

A.



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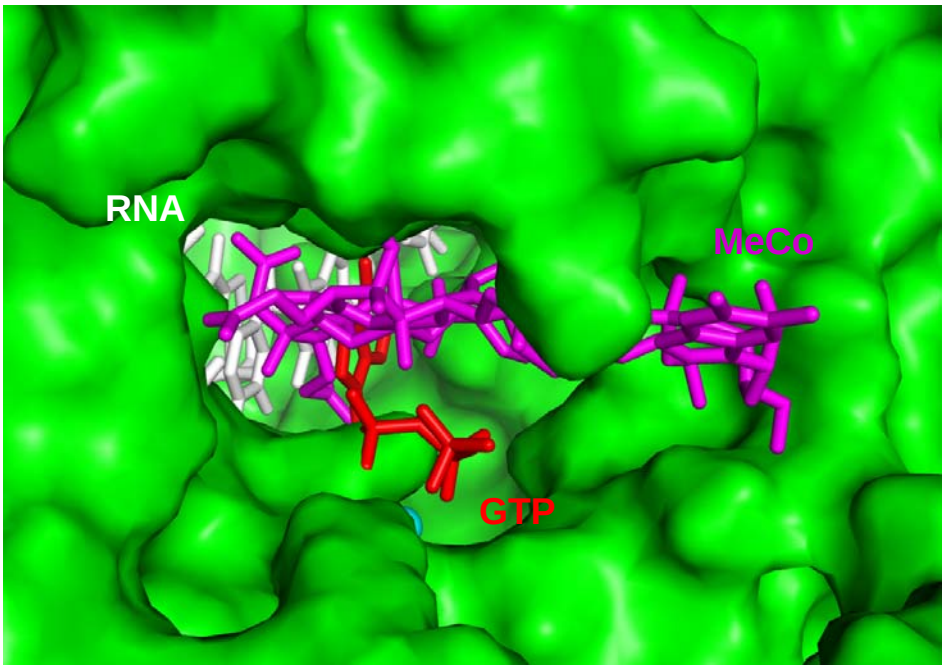

Figure 5: Binding site of methylcobalamin (MeCo) overlaps with that of incoming nucleotide. (A) Superimposition of the models of the functional ternary complex (cyan) and that of SCV2-nsp12:methylcobalamin (green) is displayed. The protein chains are shown in ribbon representation. The catalytic residues (cyan), incoming GTP (red) and methylcobalamin (magenta) are shown in stick representation and the cofactor ions are shown as spheres (B) The surface of the protein molecule is displayed and the RNA, incoming nucleotide, and methylcobalamin are shown in stick representation and coloured white, red and magenta, respectively. The vitamin B12 binding site overlaps with that of the incoming nucleotide and the terminal primer nucleotide.

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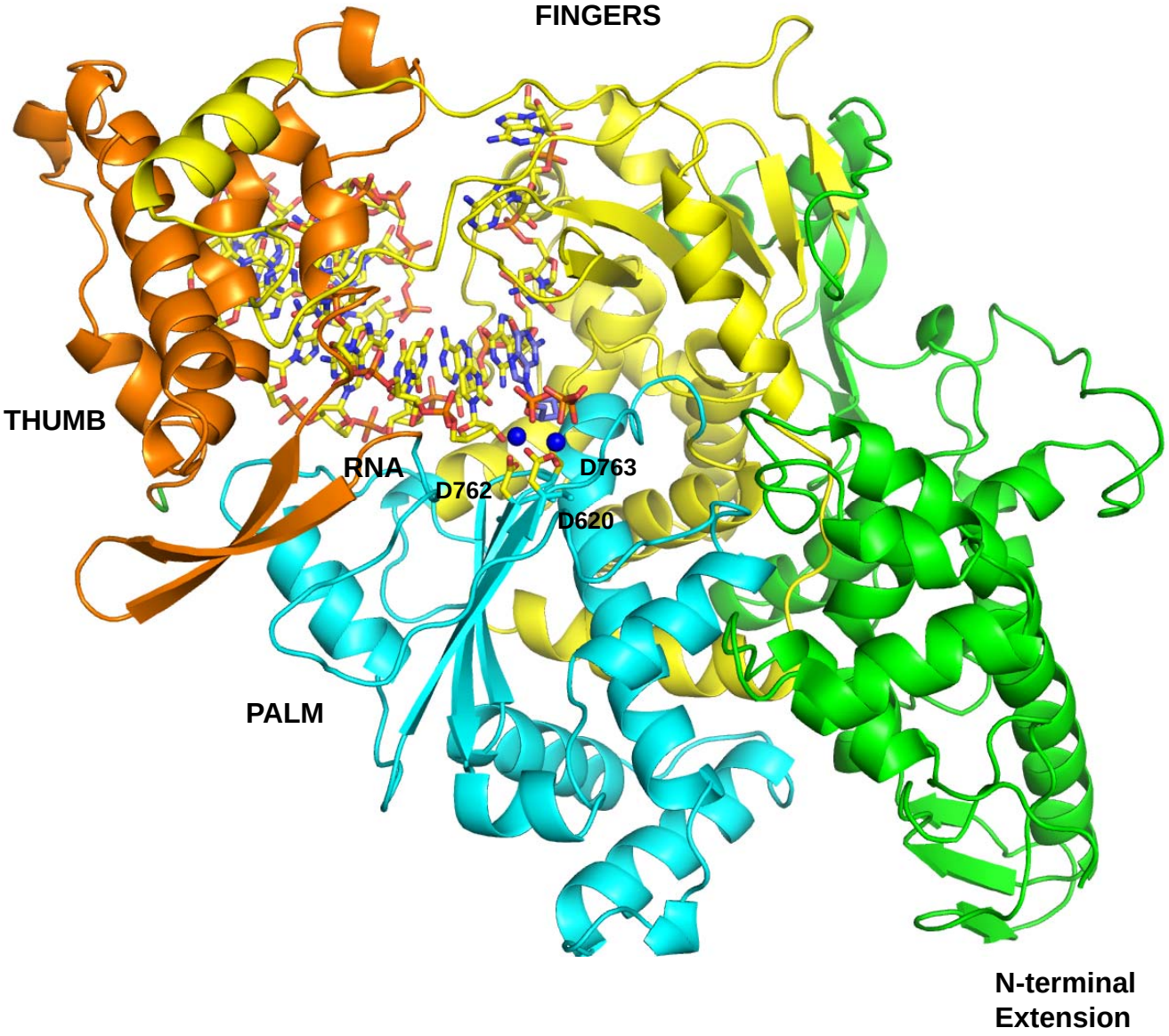

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