Journal of Experimental Biology and Agricultural Sciences

<http://www.jebas.org>

ISSN No. 2320 – 8694

FBDD: *In-silico* STRATEGY TO INHIBIT M^{PRO} ACTIVITY USING DRUGS FROM PREVIOUS OUTBREAKS

Gauravi N Trivedi^{1*}, Janhavi T Karlekar², Khushbu Dhimmar¹, Hetalkumar Panchal¹

¹Post Graduate Department of Biosciences, Centre of Advanced Study in Bioresource Technology Sardar Patel University, Satellite campus, Bakrol-Vadatal road, Bakrol - 388315, Anand, Gujarat, India.

²Indukaka Ipcowala Centre for Interdisciplinary Studies in Science and Technology Sardar Patel University, Nr. Bus Stop, B/h Shastri Maidan, Vallabh Vidyanagar – 388120, Gujarat, India.

Received – March 27, 2021; Revision – June 10, 2021; Accepted – July 11, 2021

Available Online – August 30, 2021

DOI: [http://dx.doi.org/10.18006/2021.9\(4\).472.480](http://dx.doi.org/10.18006/2021.9(4).472.480)

KEYWORDS

Main protease

M^{PRO}

FBDD

Binding Affinity

Lead-like

ABSTRACT

Main protease (M^{PRO}) and Spike (S) proteins are said potential drug targets of COVID-19. Pneumonia like respiratory illness caused by SARS-CoV-2 is spreading rapidly due to its replication and transmission rate. Protease is the protein that is involved in both replication and transcription. Since CoV-2 shares, genomic similarity with CoV and MERS-CoV, drugs from previous outbreaks are used as primary treatment of the disease. *In-silico* drug development strategies are said to be faster and effective than *in-vitro* with a lesser amount of risk factors. Fragment Based Drug Designing (FBDD), also known as rational drug design in which a potential target protein is selected and docked with a lead-like molecule that eventually leads to drug development. Nine (9) drugs that are currently being used to treat patients of coronavirus were selected in this study from the latest literature review and fragmented as per rules followed by crosslinking of drug fragments using editor tools. These native drugs and synthesized drugs were then docked against the main protease. Results of the study revealed that one of the crosslinked lead-like compounds showed a higher binding affinity (ΔG) more than any of the native compounds. Further, the results of this study suggested that the combination of potential drugs can be an effective way to develop new drugs to treat a deadly disease.

* Corresponding author

E-mail: gauravitrivedi@gmail.com (Gauravi N Trivedi)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
(<http://www.horizonpublisherindia.in/>).
All rights reserved.

All the articles published by [Journal of Experimental Biology and Agricultural Sciences](#) are licensed under a [Creative Commons Attribution-NonCommercial 4.0 International License](#) Based on a work at www.jebas.org.

1 Introduction

The novel coronavirus 2019 (nCoV-19) has been reported as a causative agent for the global pandemic in the year 2020 for spreading pneumonia-like infection among people by direct or indirect contact (Li & Clercq, 2020). This novel species of coronavirus shows symptoms like Severe Acute Respiratory Syndrome (SARS), hence also known as SARS-CoV-2 (Hui et al., 2020). The transmission rate of SARS-CoV-2 containing 30,000 nucleotides is reportedly higher than SARS-CoV (responsible for Severe Acute Respiratory Syndrome) and MERS-CoV (responsible for Middle East Respiratory Syndrome) as the reproduction number (R_0) of nCoV-19 ranges from 1.4 to 6.9 (Liu et al., 2020). Respiratory illness caused by SARS-CoV-2 leads to multiple organ failures that contagiously transmits the disease from human to human and has raised the need for the development of drug or vaccine (Panda et al., 2020). Proteins, essential for the replication are encoded by the replicase gene (Yang et al., 2006) and about two-third of the genome encodes for two overlapping polyproteins (i) pp1a (Replicase 1a, ~450kD) and (ii) pp1ab (Replicase 1ab, ~750kD) (Anand et al., 2003). These polyproteins undergo extensive proteolytic processing by viral proteases to produce multiple functional subunits that eventually form replicase complex to carry out replication and transcription (Sujinder et al., 2006). There are few therapeutic drugs (repurposed) and vaccines are available to date that can be used to prevent the damage of organs and control symptoms caused by SARS-CoV-2 (Li & Clercq, 2020). The need for vaccine or drug development has now become a necessity to get relief from this pandemic situation. Drug-like molecules are designed to bind, interact and modulate the activity of specific biological receptors like proteins or enzymes which help in the treatment of the disease. Drug designing includes target identification, target validation, lead identification, lead optimization and preclinical pharmacology and toxicology. A literature survey is one of the options for target identification. Once the target is identified, it is followed by ligand identification that interacts with the target in the same manner. Target validation is the last step to check the biological responses of the interaction. Leads are the chemical compounds that interact with proteins receptors to modulate and alter their actions. These chemical compounds must exceed a specific potency threshold against the target. Virtual libraries are the most important source of these lead compounds from where anybody can screen the leads of their interest. These virtual libraries are natural product libraries, peptides libraries or they can be carbohydrates libraries that are created by combinatorial chemistry. Lead optimization can be done by using Ligand Based Drug Designing (LBDD) and Structure-Based Drug Designing (SBDD). Indirect drug designing or LBDD is an approach where it is not necessary to have information about the 2D or 3D structure of the interested target (Sliwoski et al., 2014). LBDD works on the similar property principle which states that similar structures are likely to have similar

properties. Knowledge of chemical compounds that binds to the biological receptor is the key concept to the LBDD which ultimately leads to derive receptor by pharmacophore approach or Quantitative Structure-Activity Relationship (QSAR) (Sliwoski et al., 2014). If ligands are unknown and receptor structure is also unknown then 3D structures can be derived and generated by combinatorial chemistry. SBDD is also known as direct designing and target-based drug designing where the 3D structure of target protein is available through X-ray crystallography or NMR spectroscopy while in absence of 3D target, homology modelling can be done (Aparoy et al., 2012). Identification of ligands for the protein receptor through the database is one method for ligand identification in SBDD. De novo design of the ligands based on the structure of the binding pocket is the second parameter to keep in mind. The third approach to evaluate known ligands is by optimization of proposed ligands within the binding pocket of the target. Fragment based drug designing (FBDD) and Selective Optimization Side Activity (SOSA) are the other two approaches that are being used to design drugs with higher binding affinity and higher effectiveness. Drug-like ligands which bind to the target receptor are fragmented. Fragments must obey the Lipinski Rule of 3 (RO3) which states that the molecular weight of a fragment should not exceed 300 Da, the number of hydrogen donors and acceptors must be ≤ 3 and the value of logP must be ≤ 3 (Kirsch et al., 2019). After the fragment optimization stage, the fragments library is generated from where fragments can be derived and crosslinked with the help of linkers. Lipinski rule of 5 (RO5) is applied while merging and linking of fragments (Bienstock, 2011). Orally active drugs can not violate more than one RO5 (molecular weight ≤ 500 Da, no more than 5 hydrogen donors, no more than 10 hydrogen bond acceptors, $\log P \leq 5$) (Kirsch et al., 2019). Selective Optimization Side Activity is another approach for drug designing which uses drugs that are previously been tested for their bioactivity and toxicity (Jonckers, 2015). The selection of drugs or original chemical compounds reduced the rate of failure as it has already been approved and passed the toxicity test. This also reduces the number of hits in the drug library as it only has efficient drugs to work with (Langer & Wermuth, 2012). SOSA approach mainly works on two principles (i) creation of a smart library where drugs are carefully chosen (bioavailability and toxicity studies are already performed) (ii) Optimization of hits using traditional, or combinatorial chemistry to increase the affinity for the new target (Langer & Wermuth, 2012). The main objective of the SOSA approach is to design analogues of the original drug hit molecules to transform the side activity to the main effect (Wermuth, 2004).

1.1 CoV Main Protease

Liu et al. (2020) have successfully crystallized and structured M^{pro} from SARS-CoV-2 which is a potential target for the inhibition of CoV replication. The protein was then deposited into the Protein Data Bank and was made available to the public. M^{pro} is a

homodimeric protein with two subunits related by a crystallographic 2- fold symmetry axis. Each subunit has a length of 306 residues and it is formed mainly by 3 domains (Gimeno et al., 2020). Domain I having 8 to 100 residues and Domain II having 101 to 184 residues that share the same fold and has antiparallel six-stranded β - barrels which together resemble the structure of chymotrypsin. Domain III (201 to 306) contains 5 α - helices which are arranged into a largely antiparallel globular cluster (Hilgenfeld et al., 2006). The active site contains a cysteine-histidine catalytic dyad which is located in the cleft between domain I and II, while domain II and III are connected by the long loop formed by residue from 185-200. Domain III is involved in regulating M^{pro} dimerization which is essential for M^{pro} activity (Gimeno et al., 2020). In the crystal structure of coronavirus main proteases, the enzyme exists as dimer where 1-7 residues of N- terminal is termed as 'N-Finger', which are accommodated between two monomer units. A super active octamer form of M^{pro} has also crystallized and studied which is highly active at lower protein concentrations (Rani et al., 2020). Repurposing drugs from previous outbreaks, inhibition of vital proteins and enzymes (S, Replication Binding Domain and M^{pro}) and immune informatics for designing epitopes of MHC class I antigens for adaptive immunity can be promising *in-silico* strategies for the treatment of SARS-CoV-2. The study aims to design a drug for the main protease responsible for both replication and transmission of the coronavirus from the reported repurposed drugs having the higher binding capacity and affinity with less toxicity using computational biology methods like fragment-based drug discovery.

2 Materials and Methods

2.1 Selection of target protein

Spike (S) protein and M^{pro} are key proteins to target SARS-CoV-2 as S proteins are glycoproteins for therapeutic diagnostics that contain Receptor Binding Domain (RBD) and interacts with the peptide domain of Angiotensin-Converting Enzyme 2 (ACE2). M^{pro} is another vital enzyme that mediates viral replication and transcription together and becomes a potential target.

2.2 Protein Data Bank

Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) (<https://www.rcsb.org/>) maintains experimentally determined 3D structures of proteins and nucleic acids. The data is typically submitted by scientists obtained from X-ray crystallography, NMR Spectroscopy and cryo-electron microscopy. Since M^{pro} (PDB Id: 6LU7) is a potent target for SARS-CoV-2, in the current study 3D structure was retrieved from PDBin .pdb format.

2.3 Combinatorial library construction and Ligand preparation

A combinatorial library of ligands was constructed using a fragment based drug designing (FBDD) approach to target SARS-CoV-2.

2.4 PubChem

PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) is a database for chemical molecules and their biological activities. It is maintained by National Centre for Biotechnology Information (NCBI), a component of the National Library of Medicine (NLM) and part of the National Institute of Health (NIH) (Hilgenfeldt, 2008).

PubChem was used to search for the potent drugs reported for their antiviral bioactivities. Their three-dimensional structures were obtained in .sdf file format. Native compounds used in present study for *in-silico* drug designing are lopinavir (CID_92727), nelfinavir (CID_64143), kaempferol (CID_5280863), hydroxychloroquine (CID_3652), favipiravir (CID_492405), dexamethasone (CID_5743), remdesivir (CID_121304016), pentoxifylline (CID_4740) and quercetin (CID_5280343). Here in this article, we used abbreviations for the drugs that are lopi for lopinavir, nel for nelfinavir, kaemp for kaempferol, hcq for hydroxychloroquine, fav for favipiravir, dexa for dexamethasone, rem for remdesivir, ptx for pentoxifylline and que for quercetin. Drug-like properties for the above compounds were also checked using Lipinski RO5 and SwissADME (<http://www.swissadme.ch/>) which is a physicochemical descriptor and calculates ADME (Absorption, Distribution, Metabolism, Excretion) property for chemical compounds.

2.5 Ligand library generation using FBDD approach

Obtained drugs from PubChem were then fragmented using ChemSketch, a molecular editor that has a variety of tools and features to create and modify chemical structures. One can also view three-dimensional structures of modelled two-dimensional structures to understand the structure of chemical bonds and the nature of the functional bonds. Lipinski RO3 was kept in mind while the fragmentation process and fragment library was created. These small chemical fragments were then crosslinked with each other keeping Lipinski Rule of 5 (RO5) in mind which also states that orally active drugs should not violate more than one rule of RO5. These crosslinked fragments were then screened based on their pharmacokinetics properties including ADME using SwissADME (Antoine et al., 2017).

2.6 Drug-likeness property prediction using Swiss ADME

Swiss ADME (<http://www.swissadme.ch/>) is maintained by the Swiss Institute of Bioinformatics (SIB) which is an online platform

to compute physicochemical properties including ADME, pharmacokinetics properties and drug-like nature of a single or multiple chemical compounds at a time (Antoine et al., 2017). Topological Polar Surface Area (TPSA) is a surface sum over all polar atoms or molecules such as oxygen and nitrogen including their attachment with hydrogen. TPSA is used for the optimization of the drug's ability to permeate the cell in medicinal chemistry. TPSA > 140 angstroms squared is considered as poor permeability to the membrane (Antoine et al., 2017). Crosslinked drugs were then studied in Swiss ADME and filtered based on RO5 and TPSA values.

2.7 Protein – Ligand Docking using Autodock Vina

Target protein and synthesized ligands were then prepared for docking studies. Docking studies were done using Autodock Vina to know how efficiently ligands of virtual library binds to the receptor. The motive is to get an idea about the confirmation and binding affinity of drug-like molecules towards the target protein (Park et al., 2006). Docking is done to find a lead from a drug-like molecule which can be further used for drug development. Autodock Vina supports .pdbqt file format for both protein and ligand and is a successor version of Autodock 4 that is faster and accurate. Autodock Vina automatically generates grid files for the receptor and gives transparent results to users by multithreading on multicore machines (Park et al., 2006; Trott & Olson, 2009). 6LU7 and crosslinked drug-like molecules were then converted into .pdbqt file format and docked into the software to analyze their binding mode and binding affinity. Since Autodock Vina is GUI based software, docking results saved in .pdbqt format can be visualized in PyMol.

2.8 Visualization of docking results

PyMol is a molecular visualization software commercialized by Schrödinger, Inc (Honegger, 2017). The result from Autodock Vina was loaded in PyMol along with protein structure and were saved in .pdb file format. These results.pdb structures were opened in LIGPLOT. It automatically generates two-dimensional representation of docking output. LIGPLOT gives information about bonds and bond lengths between ligand and amino acid from the grid of the receptor (Wallace et al., 1995). After the visualization of the binding ligand to the receptor, results from Autodock Vina and Ligplot were analyzed.

3 Results

Following the method for target-based drug designing, the three-dimensional structure of 6LU7 (M^{pro}) was downloaded from Protein Data Bank. M^{pro} of COVID-19 has 96% similarity with M^{pro} of SARS-CoV which is essential for proteolytic maturation and referred to as a potential target. M^{pro} structure is of 34.51 KDa and containing 312 amino acid residues and two chains (Figure 1).

Repurposing drugs from previous outbreaks is one of the strategies used to treat people suffering from COVID-19. By following the second step of methodology, virtual screening of potential drugs from literature reading were downloaded from PubChem in .sdf file format. Some of the selected drugs are mentioned in figure 2.

Figure 2 contains the name, chemical formula and two-dimensional structure of potent drugs which were selected for rational drug designing against the target of our interest to inhibit enzyme activity. These drug candidates were then fragmented without violating Lipinski RO3 to generate a fragment library. These fragments were crosslinked with each other without violating the Lipinski Rule of 5.

Structures given in figure 3 are the illustration of 3D crosslinked chemical compounds whose nomenclature was done by taking the first three to five letters from the native name of the drug from which they are made. These drug-like compounds were then used to create a virtual library for the docking purpose against M^{pro}. Docking of these chemical compounds was performed using Autodock Vina and binding affinity was analyzed by given score (Table 1).

Docking results output.pdbqt files were then loaded and visualized into PyMOL, macromolecule visualization software as shown in figure 4 to know the binding pattern of crosslinked compounds into the binding pocket of the target protein.

The best of three docking results were then further visualized in Ligplot for detailed information about bond type between ligand and receptor, bond length and amino acid involved in the formation of bonds.

Figure 5 (A) and (B) shows that the crosslinked chemical compound has VAL104, ARG105, ILE106, GLN110, THR111, ASN151, ILE249, PRO252, PRO293, PHE294 and VAL297 in its active site of the receptor protein where ligand forms a hydrogen bond with GLN110 with 3.06 bond length.

Figure 1 Three dimensional structure of M^{pro} (6LU7) Retrieved from RCSB PDB (<https://www.rcsb.org/>)

Figure 2 Potent drug candidates / repurposed drugs for the treatment of COVID-19 Downloaded from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>)

Hcq_Lopi_Kaemp_1

Hcq_Lopi_Kaemp_2

Hcq_Lopi_Kaemp_3

Figure 4 Binding of crosslinked compounds (Hcq_Lopi_Kaemp 1/2/3) with Mpro (6LU7) visualized in PyMOL

Figure 3 3D illustration of crosslinked chemical compounds for the treatment of COVID-19 designed using FBDD approach

Figure 5A Two dimensional illustration of Hcq_Lopi_Kaemp_3 with Mpro using Ligplot

4 Discussion and Conclusions

Respiratory illness caused by SARS-CoV-2 named COVID-19 has emerged as a global pandemic and has no therapeutic drugs available to date. Drugs from previous outbreaks are used as primary treatment of COVID-19 to prevent patients from organ failure. Hence, the need for drugs or vaccines is arising day by day as COVID-19 is transmitting rapidly because of its replication cycle (Li & Clercq, 2020). The main protease is the key protein to target for rational drug design as it is involved in both replication and transcription. The current study filtered out 9 potent drugs

Figure 5B Three dimensional illustration of result obtained from Ligplot using PyMOL connectivity

which are being used to treat patients of COVID-19 from previous outbreaks and they are lopinavir, nelfinavir, kaempferol, hydroxychloroquine, favipiravir, dexamethasone, remdesivir, pentoxifylline and quercetin. Fragment based drug designing is considered as a faster and effective way to design or develop a drug (Langer & Wermuth, 2012). Fragments of the native compounds crosslinked with each other and as per our study, lopinavir (lopi) has the highest binding affinity score -8.7 Kcal/mol and hydroxychloroquine has the lowest binding score value which is -5.2 Kcal/mol (Table 1). When lopinavir was crosslinked with two other drugs hydroxychloroquine (hcq) and kaempferol

Table 1 Docking scores of repurposed drugs and crosslinked compounds using Autodock Vina

Potent Drugs	Affinity (kcal/mol)	Crosslinked Drugs	Affinity (kcal/mol)
Lopinavir	-8.7	Lopi_Rem_HCQ	-7.9
Nelfinavir	-8.1	Lopi_Rem_Ptx	-6.7
Kaempferol	-6.8	Que_Lopi_Rem	-6.1
Hydroxychloroquine	-5.2	Que_Hcq_Kaemp	-7.1
Favipiravir	-5.7	Que_Hcq_Lopi	-7
Dexamethasone	-6.8	Kaemp_Lopi_Rem	-6.3
Remdesivir	-7	Kaemp_Lopi_Hcq	-8.1
Pentoxifylline	-5.5	Fav_Lopi_Hcq	-7.1
Quercetin	-8.4	Fav_dexa_Lopi	-7.5
		Hcq_Lopi_Kaemp_1	-8.8
		Hcq_lopi_Kaemp_2	-9
		Hcq_Lopi_Kaemp_3	-9.2

(kaemp), it increases its binding affinity towards M^{pro}. Hcq_Lopi_Kaemp was first crosslinked and docked with M^{pro} which gave a higher binding score of -8.8 Kcal/mol. Three analogues of the same combination were designed and gave the best docking scores where the second analog of Hcq_Lopi_Kaemp showed -9.0 Kcal/mol binding affinity while the third analog of the same combination showed the highest of all binding affinity towards M^{pro} that is -9.2 Kcal/mol (Table 1). These three drug-like compounds do not violate more than one rule of Lipinski RO5 (Bienstock, 2011). Hence, they can be considered as orally active drugs. This combination of drugs has a stronger binding affinity than any of the native drug compounds and forms a hydrogen bond of 3.06 bond length with GLN110 from the pocket of the M^{pro} receptor. From the results of our FBDD approach, it can be concluded that computer-aided drug designing is the fastest and easier way by which a drug-like molecule can lead to a lead-like molecule and eventually to drug development.

Conflict of Interest

The authors declare no conflict of interest.

References

- Anand K, Ziebuhr J, Wadhvani P, Mesters JR, Hilgenfeld R (2003) Coronavirus Main Proteinase (3CL^{pro}) Structure: Basis for Design of Anti-SARS Drugs. *Science* 300(5626): 1763-1767.
- Antoine D, Olivier M and Vincent Z (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports* 7(1): 42717.
- Aparoy P, Reddy KK, Reddanna P (2012) Structure and ligand based drug design strategies in the development of novel 5- LOX inhibitors. *Current Medicinal Chemistry* 19(22):3763-78.
- Bienstock RJ (2011) Overview: Fragment-Based Drug Design. Library Design, Search Methods, and Applications of Fragment-Based Drug Design ACS Symposium Series; American Chemical Society: Washington, DC, Chapter 1, Pp 1-26.
- Gimeno A, Mestres-Truyol J, Ojeda-Montes MJ, Macip G, Saldivar-Espinoza B, Cereto-Massagué A, Pujadas G, Garcia-Vallvé S (2020) Prediction of Novel Inhibitors of the Main Protease (M-pro) of SARS-CoV-2 through Consensus Docking and Drug Reposition. *International Journal of Molecular Sciences* 21(11): 3793.
- Hilgenfeld R, Anand K, Mesters JR, Rao Z, Shen X, Jiang H, Tan J, Verschueren KH (2006) Structure and dynamics of SARS coronavirus main proteinase (Mpro). *Advances in Experimental Medicine and Biology* 91: 581-585.
- Honegger A (2017) Introduction to PyMOL DOI: 10.13140/RG.2.2.19625.80483.
- Hui DS, I Azhar E, Madani TA, Ntoumi F, Kock R, Dar O, Ippolito G, Mchugh TD, Memish ZA, Drosten C, Zumla A, Petersen E (2020) The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health — The latest 2019 novel coronavirus outbreak in Wuhan, China. *International Journal of Infectious Diseases* 91: 264-266.
- Ihlenfeldt W (2008) A virtual file system for the PubChem chemical structure and bioassay database. *Chemistry Central Journal* 2: 26.
- Jonkers T (2015) Selective Optimization of Side Activities (SOSA) in Drug Discovery. *The Practice of Medicinal Chemistry: Fourth Edition*, Turnhoutseweg, Beerse, Belgium.
- Kirsch P, Hartman AM, Hirsch AKH, Empting M (2019) Concepts and Core Principles of Fragment-Based Drug Design. *Molecules* 24(23): 4309.
- Langer T, Wermuth CG (2012) Selective Optimization of Side Activities (SOSA): A Promising way for Drug Discovery. *Polypharmacology in Drug Discovery: First Edition*, Wiley Online Library, Ch 11, 227-243.
- Li G, De Clercq E (2020) Therapeutic options for the 2019 novel coronavirus (2019-nCoV). *Nature Reviews Drug Discovery* 19: 19-20.
- Liu Y, Gayle AA, Wilder-Smith A, Rocklöv J (2020) The reproductive number of COVID-19 is higher compared to SARS coronavirus. *Journal of travel medicine* 27:1-4.
- Panda PK, Arul MN, Patel P, Verma SK, Luo W, Rubahn HG, Mishra YK, Suar M, Ahuja R (2020) Structure-based drug designing and immunoinformatics approach for SARS-CoV-2. *Science Advances* 6:1-38.
- Park H, Lee J, Lee S (2006) Critical Assessment of the Automated AutoDock as a New Docking Tool for Virtual Screening. *PROTEINS: Structure, Function, and Bioinformatics* 65:549–554.
- Rani R, Singh A, Pareek A, Tomar S (2020) In silico Guided Drug Repurposing to Combat SARS-CoV-2 by Targeting M^{pro}, the key virus specific protease. *ChemRxiv* 10.26434/chemrxiv.12030345.v1.
- Sliwoski G, Kothiwale S, Meiler J, Lowe EW Jr. (2014) Computational methods in drug discovery. *Beilstein Journal of Organic Chemistry* 12: 2694-2718.
- Snijder EJ, van der Meer Y, Zevenhoven-Dobbe J, Onderwater JJ, van der Meulen J, Koerten HK, Mommaas AM (2006)

- Ultrastructure and Origin of Membrane Vesicles Associated with the Severe Acute Respiratory Syndrome Coronavirus Replication Complex. *Journal of Virology* 80: 5927-5940.
- Trott O, Olson AJ (2009) AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. *Journal of Computational Chemistry* 31(2):455-61.
- Wallace AC, Laskowski RA, Thornton JM (1995) LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions Clean up structure. *Protein Engineering* 8: 127-134.
- Wermuth CG (2004) Selective Optimization of Side Activities: Another Way for Drug Discovery. *Journal of Medicinal Chemistry* 47(6):1303-14.
- Yang H, Bartlam M, Rao Z (2006) Drug Design Targeting the Main Protease, the Achilles' Heel of Coronaviruses. *Current Pharmaceutical Design* 12(35): 4573-90.