High-throughput Virtual Screening Web Service Development for SARS-CoV-2 Drug Design

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***Abstract* - The available structures of viral proteins and RNA molecules related to SARS-CoV-2 are used to screen and design a new set of drugs using the commercial databases and molecular docking protocols. The selected molecules are then studied further using molecular dynamics. Based on our earlier experiences we can target proteases, enzymes in DNA and RNA metabolism, and protein—protein interactions. In this paper we describe the planned research and development efforts for efficient screening and design of new drugs. Prior to the screening campaign, we will develop new open-source computational infrastructure, with two major outcomes. A new database containing all commercially available small-molecule ligands will be developed. A docking server with a web-based user interface will be developed and interfaced with the compound database. The docking server will use the database for sourcing of the molecules for the high-throughput virtual screening. Our approach offers major advantages that can bypass the problems that have traditionally plagued the pharmaceutical industry: our protocols are faster, cheaper, versatile, and offer minimal risks. We are developing new drugs using commercial databases, which allows us to buy the lead compounds for affordable prices that can bypass expensive and slow organic synthesis protocols.**

Keywords* - *database modeling; high-throughput virtual screening; molecular dynamics; high performance computing

I. INTRODUCTION

Viral epidemics pose an ongoing threat to public health and will continue to pose an unknown risk both to the human well-being and robustness of the world economy. Even with all scientific advancement and improvements of private, national and global healthcare systems during the 20th and early 21st century, humankind still lacks the meaning of dealing with emergent viral strains. Both human immune systems and the global pharmaceutical industry have means to neutralize only the viral strains that previously emerged in the population, and even then, quarantine and various other population isolation systems might be the only solution. One such example is the SARS-CoV-1 virus, the precursor of the SARS-Cov-2 virus responsible for the current epidemic and global shutdown. As of today, there is still no vaccine or drug that affects the SARS-CoV-1 virus, a virus that caused the SARS epidemic that officially ended on June 5th, 2003.

Viral pandemics regularly occurred during the 21st century, the most notable ones being the SARS epidemic (2002-2003), the swine flu epidemic (2009-2010), Middle East respiratory syndrome outbreaks (2012-ongoing), and current COVID-19 (2019-ongoing) pandemic. Viruses will continue to mutate and new epidemics in the future are imminent and unavoidable. Furthermore, novel vaccines cannot be developed prior to specific viral mutation and vaccine development is a process that takes time. During that time, a part of the population will inevitably be affected by whichever viral strain emerges, which is the exact current situation with the COVID-19 pandemic. No matter whether vaccine development is successful or not, and how fast its development is, there will be a large number of patients that will be in dire need of an efficient and specific drug for their ailment. Therefore, viral drug discovery remains one of the most important fields of medicinal chemistry and molecular biology, both in the academic and industrial settings.

A novel coronavirus (SARS-CoV-2) has caused 8 242 999 confirmed cases and 445 535 confirmed deaths globally, according to the COVID-19 situation report from WHO on June 18th, 2020 [1], and the numbers are still growing. Similar to the SARS-CoV outbreak in 2002, SARS-CoV-2 causes severe respiratory problems – fever, coughing, difficulties in breathing and shortage of breath are common symptoms. Human-to-human transmission occurs rapidly, and even human-to-feline contact was observed. To treat infected patients and slow this pandemic down, rapid and efficient development of highly specific antiviral drugs is of the highest urgency [2].

SARS-CoV-2 binds to a specific enzyme found on the surface of human cells. The enzyme, named angiotensin-converting enzyme 2, or ACE2 for short, lines endothelial cells in the lungs' blood vessels. The enzyme happens to be the same target for SARS-CoV-1, the virus that caused SARS.

Like other kinds of coronaviruses, SARS-CoV-2 forms a series of "spike" proteins that protrude from its core, giving it the shape of the sun's corona. Those spike proteins latch onto ACE2, which allows the virus to fuse its membrane to the cell membrane. Once inside, SARS-CoV-2 acts like any other virus – it hijacks the human cell, forcing the cell to produce many copies of the virus, spurring an infection.

A team of researchers designed a Michael acceptor inhibitor N3 using computer-aided drug design (CADD),

which can specifically inhibit multiple CoV Mpros, including those from SARS-CoV and MERS-CoV [3] – [6]. They constructed a homology model of COVID-10 Mpro and used molecular docking to see if N3 could target this new CoV Mpro. A docking pose showed that it could fit inside the substrate-binding pocket. To assess the efficacy of N3 for COVID-19 Mpro, a kinetic analysis was performed. A progress curve showed that it is a time-dependent irreversible inhibitor of this enzyme. The shape of this curve supports the mechanism of two-step irreversible inactivation. The inhibitor first associates with COVID-19 Mpro with a dissociation constant Ki; then, a stable covalent bond is formed between N3 and Mpro (E-I). The evaluation of this time-dependent inhibition requires both the equilibrium-binding constant Ki and the inactivation rate constant for covalent bond formation k3. However, N3 exhibits very potent inhibition of COVID-19 Mpro, such that measurement of Ki and k3 proved difficult. When very rapid inactivation occurs, kinetic constant kobs/[I] was utilized to evaluate the inhibition as an approximation of the pseudo-second-order rate constant (k3/Ki) [3]. The value of kobs/[I] of N3 for COVID-19 Mpro was determined to be 11,300±880 M-1s-1, suggesting this Michael acceptor has potent inhibition.

To elucidate the inhibitory mechanism of this compound, they determined the crystal structure of COVID-19 Mpro in complex with N3 to 2.1-Å resolution [7]. The crystal structure is available in the PDB protein databank as 6LU7 entry [8] (shown on Fig. 1). All residues (residues 1–306) are visible in electron density maps. Each protomer is composed of three domains. Domains I (residues 8–101) and II (residues 102–184) have an antiparallel β-barrel structure. Domain III (residues 201–303) contains five α-helices arranged into a largely antiparallel globular cluster and is connected to domain II through a long loop region (residues 185–200). COVID-19 Mpro has a Cys–His catalytic dyad, and the substrate-binding site is located in a cleft between Domain I and II. These features are similar to those of other Mpros reported previously [4], [6], [9]–[10]. The electron density map shows that N3 binds in the substrate-binding pocket in an extended conformation (Fig. 2, Fig. 3), with the inhibitor backbone atoms forming an antiparallel sheet with residues 164–168 of the long strand 155-168 on one side, and with residues 189–191 of the loop linking domains II and III.

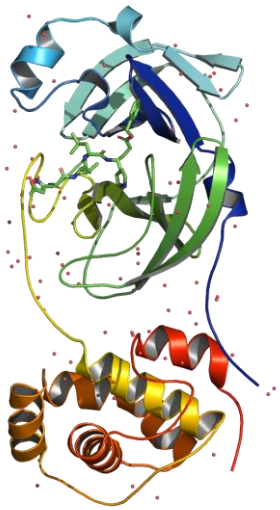

Figure 1. 6LU7 - The crystal structure of COVID-19 main protease in complex with an inhibitor N3 visualized using PyMOL [11]

II. OBJECTIVES

A. High-throughput virtual screening campaign of SARS-CoV-2 targets with commercially available compounds

N3 forms multiple hydrogen bonds with the main chain of the residues in the substrate-binding pocket, which also helps lock the inhibitor inside the substrate-binding pocket (Fig. 2).

The structure of SARC-CoV-2 Mpro in complex with N3 provides a model for rapidly identifying lead inhibitors to target SARS-CoV-2 Mpro through in silico screening. To achieve this, the existing and new molecular docking software will be combined to screen the compounds efficiently.

Virtual screening (VS) is a computational technique used in drug discovery to search libraries of ligands in order to identify those protein receptors which are most likely to bind to a drug target. VS can be followed by molecular dynamics, a computer simulation method for studying the movements of protein-ligand complexes in space over time. High-throughput virtual screening (HTVS) is a type of VS where the amount of dedicated computational resources is very high so many ligands can be checked at the same time.

B. Virtual screening of bioactive peptide and synthetic peptides against SARS-CoV-2 and ACE2 protein-protein interface

Recent crystallographic studies of the SARS-COV-2 receptor-binding domain (RBD) and full-lengths human ACE2 receptor revealed key amino acid residues at the contact interface between the two proteins and provide valuable structural information that can be leveraged for the development of disruptors specific for the SARS-CoV-2/ACE2 protein-protein interaction (PPI) [12], [13]. Small-molecule inhibitors are effective in binding to targeted binding sites, such as the aforementioned SARS-CoV-2 and N3 complex binding site but are less effective at disrupting extended protein binding interfaces [14]. Peptides, on the other hand, offer a synthetically

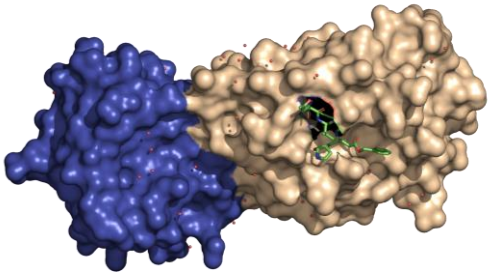

Figure 2. 6LU7 - The surface view of COVID-19 main protease in complex with an inhibitor N3 visualized using PyMOL [11]

accessible solution to disrupt protein-protein interactions by binding at interface regions containing multiple contact "hot spots" [15]. Analyzing the RBD-ACE2 co-crystal structure, we found that SARS-CoV-2-RBD/ACE2 interface spans a large elongated surface area, as is common for protein-protein interactions.

We hypothesize that disruption of the viral SARS-CoV-2-RBD and host ACE2 interaction with peptide-based binders will prevent virus entry into human cells, offering a novel opportunity for therapeutic intervention. To accomplish this, all proven bioactive peptides will be screened on the SARS-CoV-2-RBD domain using the aforementioned methodology, and the best peptides will serve as a model for the development of novel, synthetic peptides that will specifically target SARS-CoV-2-RBD surface. The peptides will then be evaluated using molecular dynamic simulations to observe specific polar and non-polar protein—protein interactions between the functional domains of SARS-CoV-2-RBD and ACE2.

C. *All-atom molecular dynamic simulations of single- and double-anti-viral therapy effect of SARS-CoV-2 binding to ACE2 target*

The co-crystal structure of SARS-CoV-2-RBD with ACE2 (PDB: 6M17, Fig 3.) will be used for the initial structure for molecular dynamic (MD) simulation for peptide binders development. Standard MD simulation procedures will be used during this work, particularly conformational exploration simulations of protein-protein docking, model refinement and testing of homology modeled SARS-CoV-2 proteins, addition and removal of ligands from HTVS campaign, mutation and modification of specific peptide chains, application of mechanical force to characterize strength of protein-protein interactions, and conformational changes of protein-protein supramolecular complex with various ligands and synthetic peptides designed to disrupt said complex.

The same MD simulations will be used for SARS-CoV-2 Mpro in complex with N3 compound (PDB: 6LU7) for the same purpose. Additionally, MD simulations of Mpro with screened ligands will be run, using the same research methodology. At the very end of this research work, MD simulations with both small-molecule inhibitors of Mpro and peptide binders on the SARS-CoV-2-RBD/ACE2 PPI region will be run to assess the effect of double anti-viral therapy.

III. RESEARCH AND DEVELOPMENT METHODOLOGY

A. *Design and development of the compound database*

The potential inhibitors, sourced from vendors of commercially available compounds, will be stored in a compound database. The existing databases were evaluated for the purpose: the freely accessible databases such as ChemSpider [16] by Royal Society of Chemistry and PubChem [17] by U.S. National Library of Medicine are not open source software that can be extended, interfaced, and changed according to the research requirements of the various projects and in particular the research and development work proposed here. Therefore, the compound database that satisfies the following requirements will be designed and developed:

- Able to hold millions of compounds
- Searchable according to name, structure, and molecular descriptors
- Able to be updated with new data from commercial databases

The compound database will be interfaced with the docking server described in the following to allow the easy screening of selected compounds.

B. *Design and development of the docking server*

The engine of the screening workflow that will be used is RxDock (formerly RiboDock(r) [18] and rDock [19]), a fast, versatile, and open-source program for docking ligands to proteins and nucleic acids developed by Vernalis R&D, University of York, University of Barcelona, RxTx, and others [20]. RxDock and its predecessor rDock were specifically designed for high-throughput virtual screening usage and are already used for HTVS of compounds that could bind to SARS-CoV-2 proteins, in particular by Galaxy [21], [22] and COVID.SI [23].

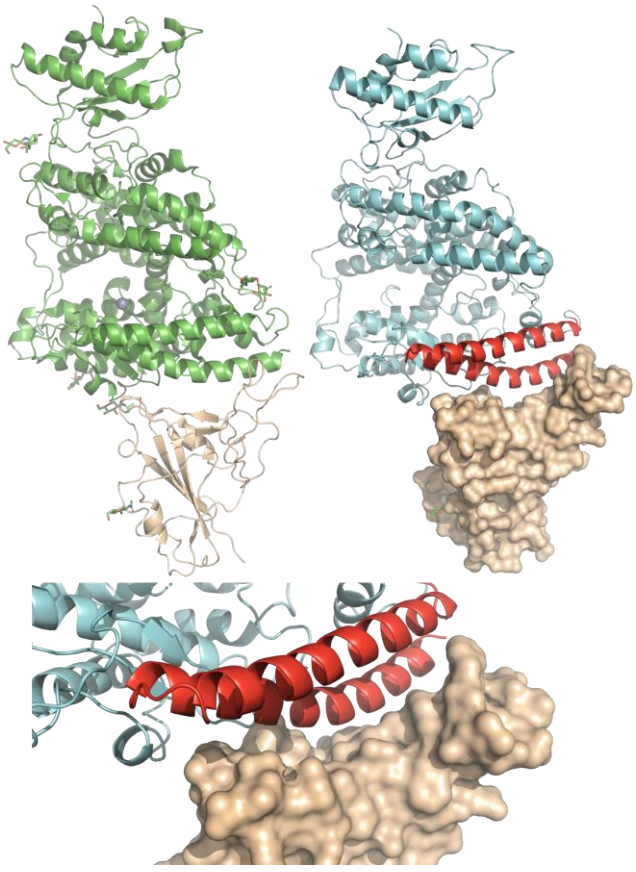

Figure 3. (Top left) SARS-CoV-2-RBD/ACE2 protease domain (PD) cryo-EM structure (PDB code: 6M17) in cartoon mode. ACE2 is colored in green, while SARS-CoV-2-RBD is colored in wheat color. (Top right) SARS-CoV-2-RBD/ACE2 protease domain (PD) cyro-EM structure. Selection of peptide fragments making key contacts colored in red. SARS-CoV-2 RBD is shown in surface mode, colored in wheat color. (Bottom) SARS-CoV-2-RBD/ACE2 key contact close-up. (All figures visualized using PyMOL [11])

Gorgulla et al. recently had a paper accepted in Nature [24] that described VirtualFlow, an approach to running HTVS campaigns on supercomputers managed by a batch system such as SLURM. Two types of tasks are performed by VirtualFlow: ligand preparation (checking the validity of the input files, a transformation of the input files to supported formats, cutting input files into pieces for multi-process execution, etc.) and virtual screening. The approach of VirtualFlow will be used for running HTVS campaigns with RxDock on Bura supercomputer. Instead of running jobs directly via scripts, a custom docking server with a web interface will be used. The interface will allow the user to specify a set of ligands (selected from the compound database) and the target site on the protein and will use a connection to Bura for scheduling docking jobs and gathering results after the jobs have finished executing for presenting them back to the user (Fig. 4).

The docking server and the compound database will be deployed at the University of Rijeka, Department of Informatics for usage by the researchers working on this problem and by the wider research community. Additionally, both the docking server and the compound database software developed in the scope of this work will be released under open-source licenses so they can be studied, improved, and deployed elsewhere by other researchers for other purposes.

C. Drug repositioning

Using newly developed infrastructure, a high-throughput virtual screening campaign of all FDA approved drugs will be performed. Crystal structures of SARS-CoV-2 Mpro structures (6LU7, 6YB7, 6M17, and any other relevant Mpro crystal structure or in silico model) be prepared accordingly before HTVS campaign itself. Hydrogens will be added, and structures will subsequently be minimized using the custom force field. The protonation state of His residues and the orientation of hydroxyl groups, Ans residues, and Gln residues will be optimized as well. Compounds from FDA approved drugs database will be protonated and 3D structure will be generated using the MMFF94s force field.

Pharmacophore approaches are one of the crucial tools in drug discovery [25]. IUPAC defines pharmacophore as "an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response" [26]. A pharmacophore model can be constructed in two ways:

- ligand-based: by overlapping a set of active molecules and deriving prevailing chemical features that are essential for their bioactivity
- structure-based: by probing possible interaction points between the macromolecular target and ligands.

Structure-based pharmacophore modeling will be performed to obtain potential structures and a subset of the database matching the structure will be used for screening.

Once the protein structures are prepared, binding pockets will be defined and calculated using the cavity search engine of RxDock. Molecular docking itself will be carried out using the RxDock docking engine, considering the ligands as flexible but treating the receptor as a rigid structure. A post-docking minimization of the most promising docking results according to the docking score will be performed using the GROMACS software suite. The best candidates will be used in all-atom MD simulations of their disruption effects of SARS-CoV-2/ACE2 enzyme binding. The systems will be explicitly solvated to perform incrementally increasing MD simulation – starting simulations with initial binders will be evaluated using 20 ns all-atom simulations, with an increase to 50, 100 and 200 ns simulations in each passing run – using GROMACS on University of Rijeka's Bura supercomputing cluster.

D. HTVS campaign of commercially available small-molecule ligands

Using the same methodology as previously described, commercially available small-molecule ligands from all major and relevant commercial vendors will be evaluated in the HTVS campaign. Three specific subsets of ligands will be defined before HTVS campaign itself:

- Molecules with similar SAR properties of known SARS-CoV-2 ligands (such as N3)
- Molecules that comply with the Lipinski Rule of Five
    - Molecules that comply with the Lipinsky Rule of Three will be specially favored
- Molecules that comply with Ghose filter.

Each subset will be evaluated independently. The best candidates from each group will be used in all-atom MD simulations of their disruption effects of SARS-CoV-2/ACE2 enzyme binding using the same MD protocol explained previously.

E. Peptide binder design

Bioactive peptide structures and descriptors will be stored in newly developed database. For simulating these

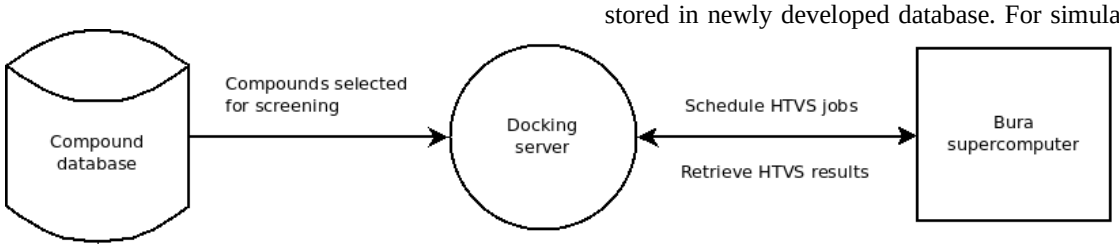

Figure 4. The interactions between the compound database, the docking server, and the Bura supercomputer

peptides inside a complex with SARS-CoV-2-RBD domain in GROMACS, usage of a99SB-disp and CHARMM36m force fields will be considered for their ability to model coil-to-structure transitions [27], [28]. Synthetic helical peptide sequence of spike-binding peptide 1 derived from the α1 helix of ACE2 peptidase domain (ACE2-PD) and synthetic peptide sequences similar to the bioactive peptides that performed best in MD simulations will also be evaluated (example MD simulation of protein—protein interactions from prior work.

IV. EXPECTED RESULTS

We are confident that we can perform full HTVS campaign of the major commercial databases on RNA-dependent RNA polymerase from SARS-CoV-2 virus, SARS-CoV-2 spike protein, protease of SARS-CoV-2 and other emerging targets an also develop the software infrastructure to make future endeavors of the same type easier. Our expected results out of HTVS campaign of FDA approved drugs and commercially available compounds (several million compounds) are couple of dozens of compounds with binding affinity lower of 1 μM.

Every molecule sequence that shows homology with the SARS-CoV-2 genes will be generated and the resulting model will be minimized using coarse-grain and all-atom MD protocols.

The effect of single- and double-anti-viral therapy will be evaluated. The effect of small-molecule ligands and peptide binding to SARS-CoV-2 targets and disruption of SAR-CoV-2/ACE2 complex (single-anti-viral therapy) and combined small-molecule ligand and peptide disruption of SARS-CoV-2/ACE2 complex will be ranked.

V. CONCLUSION AND FUTURE WORK

The authors of this paper are proposing a research and development of a novel computational infrastructure and research model for viral drug discovery and development. Reasons for proposing this research and development are: The first problem we want to address is the issue of chemical synthesis during the drug discovery phase. Chemical synthesis of novel compounds is a time-consuming process with a variable rate of success and during an ongoing epidemic/pandemic, it is a step that would best be avoided. Our solution for that is the development of a database that queries all major commercial compound databases for compounds that are available for purchase at the starting moment of a HTVS campaign.

The second issue we want to address is a lack of proper and well-documented open-source docking platforms that can easily be scaled on any hardware, from basic workstations to complex supercomputers in any of the major HPC centers, and deployed at any time by any research group without any licensing issues. We will be using RxDock as a novel docking platform specifically designed for HTVS campaigns and open-source any novel features we develop. In that way, any progress we make on developing novel drugs for the SAR-Cov-2 virus can be deployed in the future for any project by any research group on whatever (super)computer they have access to. The key component we will develop is a docking server built on top of RxDock, interfaced with compound database for sourcing of ligands and the Bura supercomputer for performing molecular docking. The web interface to the docking server will make the setup of large HTVS campaigns straightforward regardless of the device used by the user for accessing the interface.

The third and final problem we plan to solve is the discovery of novel SARS-CoV-2 drugs using infrastructure we develop. Since the main targets for viral infections are various proteases, we will utilize our extensive research experience in protease mechanism research [29]–[34] to simulate ligand-protein interactions of the most promising hit candidates from HTVS campaigns. The simulations will be run on local HPC Bura infrastructure. Insights gained during this research and development will be applied for all subsequent work with the infrastructure we develop ready to deploy at any time.

Since all parts of the proposed infrastructure are and are going to be open-source, resulting infrastructure will feature the following characteristics:

- reproducibility – all results generated on our software infrastructure will be easily repeated by any research group
- speed of setup – once developed, all software solutions will be easily deployed within a working day on any hardware by any research group or private company
- collaboration – multiple research groups will be able to collaborate using a single server setup.

Furthermore, the novel drug discovery infrastructure we develop is target-agnostic and will retain its full usefulness after this work is completed, and for any subsequent and inevitable viral epidemic. Some of the ongoing projects that will utilize this infrastructure include the development of DNA methyltransferase inhibitors, ubiquitin iso-peptidase inhibitors, and γ-secretase modulators.

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