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Darunavir Disrupts Critical Nodes in Metastable 2019-nCoV-RBD/ACE-2 Complex

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Abstract:

The transnational spread of coronavirus (2019-nCoV) first detected in Wuhan is causing global panic; thus, accelerated research into clinical intervention is of high necessity. The spike glycoprotein structure has been resolved, and its affinity to human angiotensin-converting enzyme 2 (ACE-2) has been experimentally validated. Here, using computational methods, a metastable conformation of 2019-nCoV-RBD/ACE-2 complex has been revealed and FDA-database of approved drugs have been docked into the interface. Darunavir has been discovered as high ligand affinity candidate capable of disrupting communication between 2019-nCoV-RBD and ACE-2. Darunavir, in addition to its previously known anti-HIV protease inhibitor is now repurposeable for the treatment 2019-nCoV disease acting via disruption of cellular recognition, binding and invasion.

Keywords: 2019-nCoV, Darunavir, ACE-2, Receptor Binding Domain, Metastable Conformation, FDA database.

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1. Introduction

A novel coronavirus (2019-nCoV) first detected in Wuhan, but now spreading to other nations through human-to-human contact is the causative agent of pneumonia outbreak, and death due to progressive respiratory failure [1]. To stem the global health threat it currently poses, there is a need to further investigate the biochemistry underlying its pathogenesis and tropism [2] with a view of biologics or therapeutic development. Indeed, the Ebola vaccine development program, has proven handy in fast-tracking the development of vaccine candidates since the release of its entire genome assembly [3,4].

The effort at developing small molecular weight drugs has not lagged as well, with the structural elucidation of key protein structures; including a protease in co-crystallized with an inhibitor (PDB ID: 6LU7); and its spike glycoprotein [5]

The current study focuses on the mechanism of cellular invasion and the exploration of its therapeutic potentials.

First, although phylogenetic comparison places 2019-nCoV as closest to the bat-SL-CoVZC45 and bat-SL-CoVZXC21 but alignment of the receptor-binding domain (RBD) of the 2019-nCoV strongly suggest close similarities with severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV). Subsequently, human angiotensin-converting enzyme II (ACE-2) has been proven to be the human receptor during cellular invasion of epithelial cells of oral mucosa [6].

The 2019-nCoV-RBD/ACE-2 complex proposed in this study was obtained by superimposing the spike glycoprotein with receptor-binding domain in up conformation on SARS Spike Glycoprotein - human ACE2 complex [7] as previously proposed and experimentally validated[5]. This complex was subjected to 350 ns atomistic simulation to harvest metastable conformations which was used for screening of FDA library.

2019-nCoV-RBD/ACE-2 bound to the highest affinity compound obtained from docking was subjected to further simulations to investigate the disruption of community interaction between 2019-nCoV-RBD and ACE-2.

Here, we report Darunavir as an anti-2019-nCoV disease candidate acting via disruption of viral tropism.

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2. Materials and Methods

2.1. Starting structure:

The 2019-nCoV spike glycoprotein homotrimer used in this study was the exact structure crystallized by Wrapp et al. [5] except that the up conformation was remodeled using swiss modeling server (swissmodel.expasy.org/) [8] to replace broken chains and incomplete residues. The ACE-2 coordinates were retrieved from a previously crystallized SARs-ACE-2 complex [7]. The two structures were aligned using PyMol alignment tools [9] and the 2019-nCoV-RBD coordinates were excised and added to ACE-2 coordinates. The structures of FDA library was retrieved from FDA webserver (https://www.fda.gov/drugs/drug-approvals-and-databases/drugsfda-data-files). Cheminformatic manipulation (removing all compounds with mass lesser than 200 and greater than 1700) of the library was performed using DataWarrior [10]. Molecular docking runs were performed using mcule platform (https://mcule.com/). Darunavir/2019-nCoV-RBD complex was retrieved from mcule platform for molecular dynamics simulation.

2.2. Preparation of Complex for Molecular Dynamics Simulation

HTMD notebook was used to prepare 2019-nCoV-RBD/ACE-2 atoms (charmm36 forcefield) for MD simulation using ACEMD. The parameter for Darunavir were CHARMM General Force Field (ParamChem) [11]. All conditions for equilibration and MD simulation have been previously reported by our group [12]. The Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) method for calculating the binding energies between energies have been reported [13]. 3D free energy landscape was computed using Wolfram Mathematica from *gmx sham* pre-processed protein-rmsd/radius of gyration values; network analysis were also performed using VMD network Tools program as we have previously reported [14].

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3.0. Results and Discussion

3.1. Computational Alignment of 2019-nCOV-RBD/ACE-2 Based on SARs 3D structure

We projected the space-filing model of 2019-nCoV homotrimer spike glycoprotein (up-conformation) aligned to SARS-RBD to illustrate the fit, when interacting with ACE-2; the putative receptor. (Fig. 1, *i*). On a closer look, the extended loops of the RBDs appear at an interacting distance from α1 helix of ACE2.. ACE-2 α2 helix, β3 and β4 antiparallel strands, and their connecting Loops also may also contribute to the recognition or binding of the RBDs (Fig. 1, *ii*).

A detailed mapping of the residues identified Y453, Q493, Y499, Y505, and T500 of 2019-nCoV making contacts with D30, H34, D38, N330 and K353 of ACE-2 (Fig. 1, *iii*). Incidentally, a just-reported cryo-EM structure of human ACE2-bound 2019-nCoV-RBD has equally identified these residues; making the model valid for further studies (Yan et al., 2020; pre-print: https://doi.org/10.1101/2020.02.19.956946).

3.2. Free-Energy Landscape studies identified β-sheet/Loop transition may drive binding flexibility in 2019-nCoV-RBD/ACE-2 interaction.

In the absence of a crystal structure during as the starting structure at the beginning of this study, there was a need to explore the free energy surface in order to retrieve a metastable conformation for further studies. Therefore, following 350 ns simulation, RMSD and radius of gyration variables were projected along the 3D free energy surface (Fig. 2, *i*). At the free energy values exceeding 6.0 kcal/mol, the predominant conformation sampled by the 2019-nCOV-RBD was those with most of the conformational differences were observed with the an antiparallel β-sheet (designated **β1/2**) but in free-energies of 0.0 kcal/mol(Fig. 2, *ii*), there is structural transition to loop structure (Fig. 2, *iii*). Notably, ACE-2 structure was stable in all the conformations sampled. Interestingly, the reported cryo-EM structure of human ACE2-bound 2019-nCoV-RBD did show the loop but not the anti-parallel sheet; clearly lending credence to the accuracy of the model used for the molecular docking in the next step. However, the essence of this transition may be a higher flexibility

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required to better interact with ACE-2 residues which offer limited interaction during in the starting structure. Indeed, a close-up snapshot revealed key 2019-nCoV-RDB residues previously in position not optimum for interaction has been relocated very proximal to ACE-2 (Fig. 2, *iv*).

3.3. Molecular docking of FDA Library identified darunavir and ubrogepant as 2019-nCoV-RBD/ACE-2 complex high affinity binder

FDA library of compounds have provided a rich source of resource for drug repurposing; it is known to be cheaper, rapid and safer route to developing a new therapeutics for old or emerging diseases [15]. Here, starting using a molecular weight cut-off criteria of >250 and < 1800 g/mol, about 800 unique compounds were selected for docking into 2019-nCoV-RBD/ACE-2. Interface using the most stable conformation obtained from the FES study**.** Two compounds darunavir and ubrogepant were selected based on the ligand efficiency index[16]. Both compounds have < 550 g/mol molecular weight and a docking score of ~ -11.0 kcal/mol (Fig. 3). Although both compounds exhibited a good docking score, an initial investigation of both of them showed that darunavir but not ubrogepant had an history as an anti-viral compound [17]; therefore, selected for further computational studies. First, the preference of binding was determined using MMPBSA [13] free energy values obtained from 2019-nCoV-RBD in complex with darunavir and those obtained from the candidate bound to 2019-nCoV-RBD/ACE-2. The result depicted that darunavir preferably interacts with 2019-nCoV-RBD/ACE-2 (-174.36 kJ/mol) complex not 2019-nCoV-RBD (-110.06 kj/mol) (Table 1.0).

3.4. Molecular Simulation showed darunavir ruptures 2019-nCoV-RBD/ACE-2 complex

Starting from the most darunavir docked to the metastable conformation obtained in the FES study, another 250 ns simulation was performed in comparison without the compound; following a network analysis of the trajectories, the critical interaction in darunavir-bound complex was completely ruptured between 2019-nCoV-RBD/ACE-

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2 (Fig 3, *i-iv*); whilst also reducing the network community between ACE-2 and 2019-nCoV-RBD. Since SARS-CoV gains cellular invasion by recognition and binding of its RBD with the cell surface ACE-2 and 2019-nCoV adopts the same mechanism, darunavir therefore represents a novel 2019-nCoV entry inhibitor. Some of the antiviral drugs have clinical potency as entry inhibitors [18].

4. Conclusion

2019-nCoV disease has continued to pose major threat to the world; and the case-to-fatality ratio has continued to rise. To tackle this challenge, there is a need to rapidly develop therapeutic intervention. In this study, darunavir has been discovered as novel anti-2019-nCoV disease agent acting via disruption of 2019-nCoV-RBD/ACE-2 interaction. This drug promises to alter the viral tropism and the dynamics of human-to-human transmission when tested in laboratory or clinical settings.

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Figures:

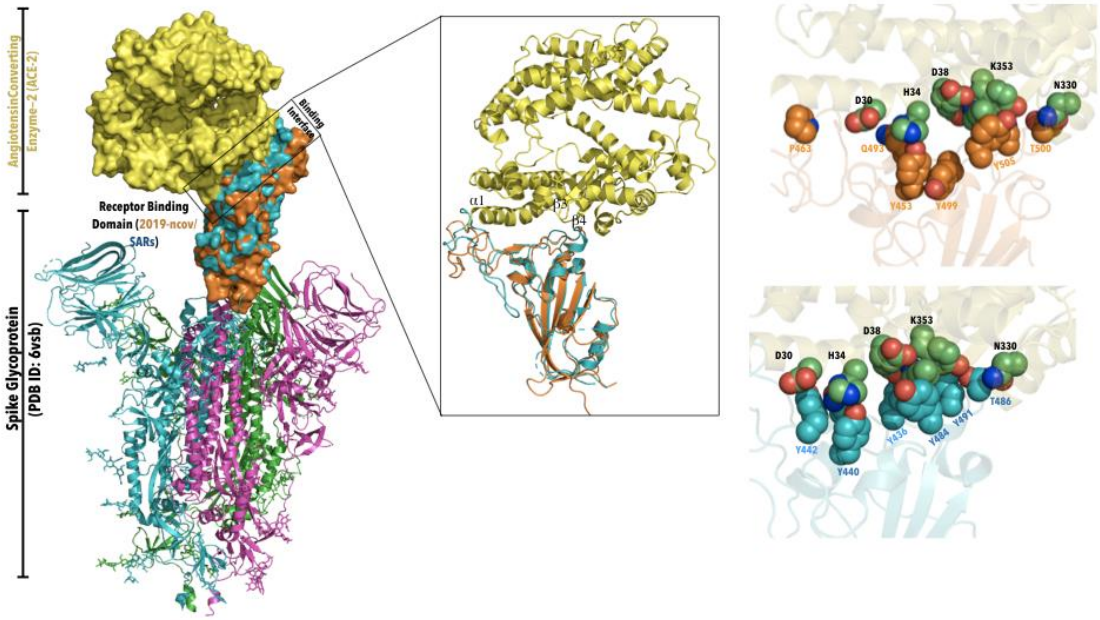

Fig. 1: Alignment of 2019-nCOV-RBD/ACE-2 Based on SARs 3D structure

Fig. 1: 3D model of 2019-nCoV in ACE-2 bound state. (*i*) Space-filling representation of 2019-nCoV-RBD/human ACE-2 (yellow). (*ii*) Cartoon representation of the RDB/ACE-2 complex showing the secondary structures of the proteins. (*iii*) A close-shot view of the complex showing the residues involved in RDB/ACE-2 interaction.

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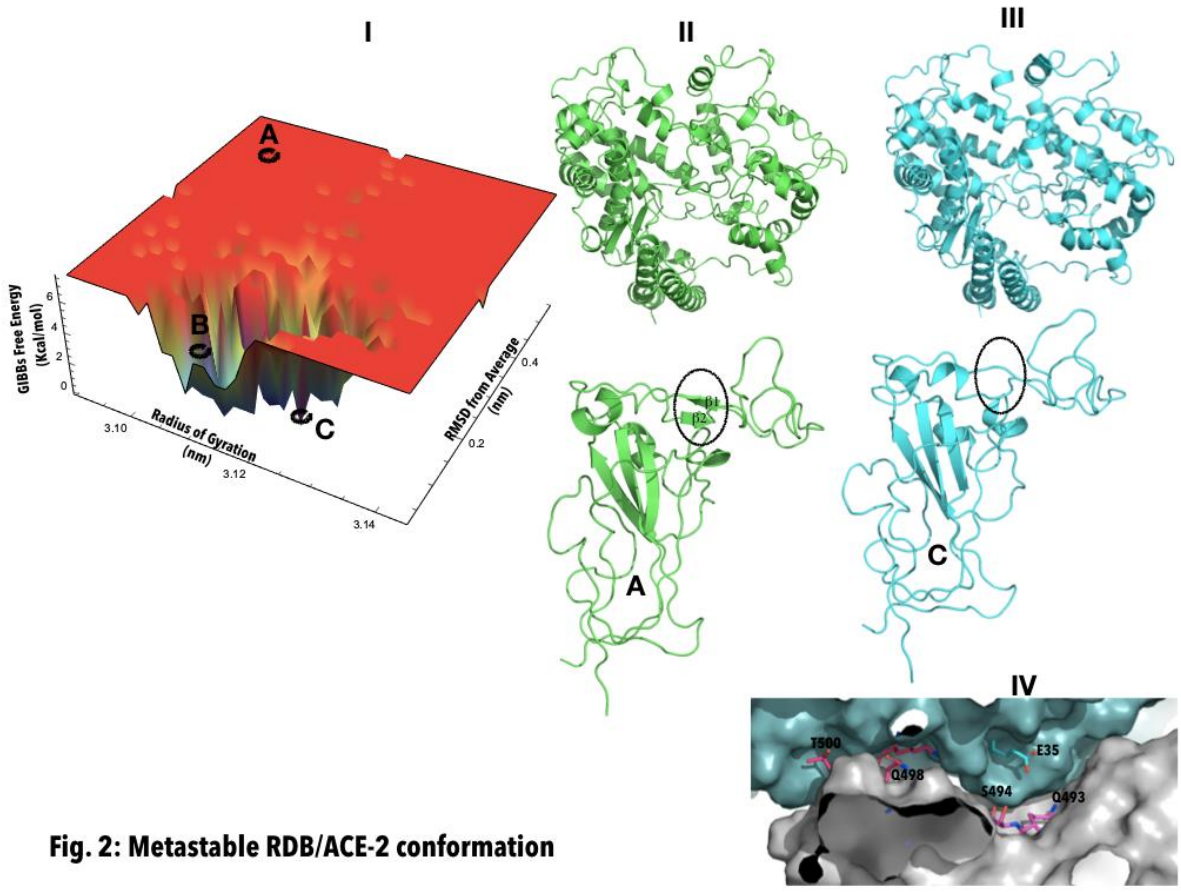

Fig. 2: Metastable RDB/ACE-2 conformation

Fig. 2: Metastable RDB/ACE-2 conformation. (*i*) Free energy surface plot from projections of radius of gyration (nm) and rmsd (nm), showing unstable (A) and highly metastable conformation (C). (*ii*) Cartoon representation of ACE-2 and RBD (green) from the unstable conformation. (*iii*) Cartoon representation of ACE-2 and RBD (green) from the metastable conformation. (iv). Surface representation of RDB/ACE-2 depicting key residues in the interface.

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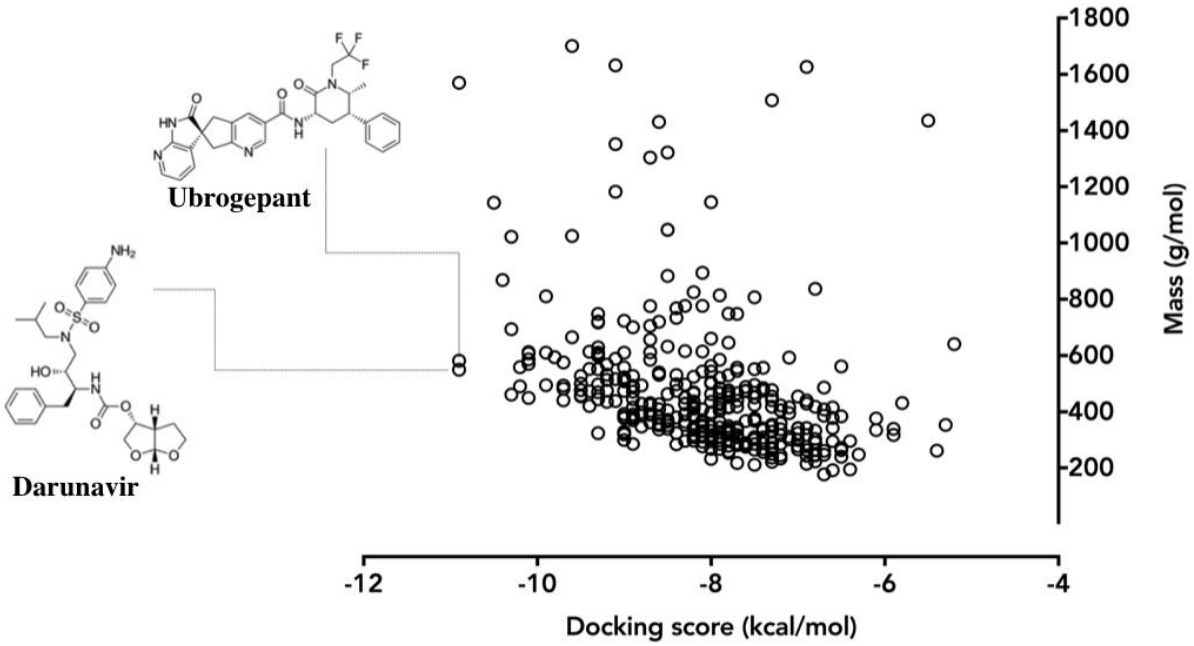

Fig. 3: Selection of candidate drugs

Fig. 3: Selection of candidate drugs: Scatter plot of the molecular mass of selected candidates in the FDA database and their docking score into the RDB/ACE-2 interface. The graphs shows darunavir and ubrogepant as potential candidates.

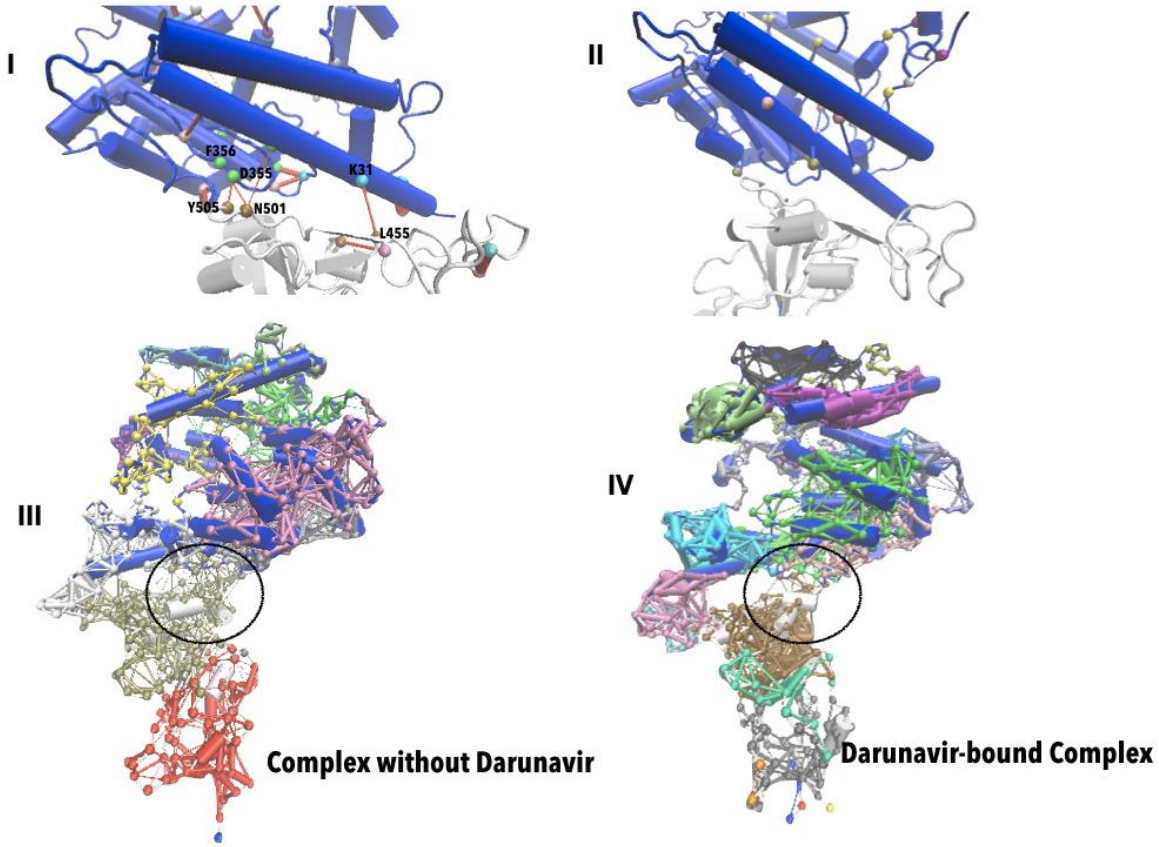

Fig. 4: Darunavir is a disruptor of RDB/ACE-2 interaction

Fig. 4: Darunavir is a disruptor of RDB/ACE-2 interaction. (*i*) critical interaction nodes in RDB/ACE-2 complex without darunavir. (*ii*) critical interaction nodes in RDB/ACE-2 complex with darunavir. (*iii*) Community network interaction in the absence of darunavir. (iv) Community network interaction in the presence of darunavir.

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