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Letter

Genetic Variability of the SARS-CoV-2 Pocketome

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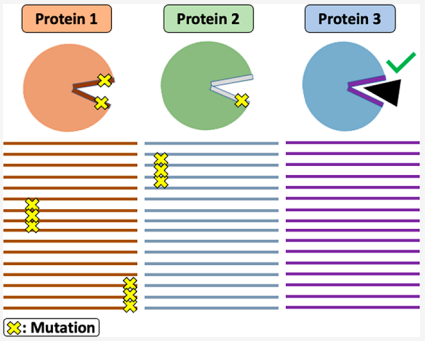
ABSTRACT: In the absence of effective treatment, COVID-19 is likely to remain a global disease burden. Compounding this threat is the near certainty that novel coronaviruses with pandemic potential will emerge in years to come. Pan-coronavirus drugs—agents active against both SARS-CoV-2 and other coronaviruses—would address both threats. A strategy to develop such broad-spectrum inhibitors is to pharmacologically target binding sites on SARS-CoV-2 proteins that are highly conserved in other known coronaviruses, the assumption being that any selective pressure to keep a site conserved across past viruses will apply to future ones. Here we systematically mapped druggable binding pockets on the experimental structure of 15 SARS-CoV-2 proteins and analyzed their variation across 27 α- and β-coronaviruses and across thousands of SARS-CoV-2 samples from COVID-19 patients. We find that the two most conserved druggable sites are a pocket overlapping the RNA binding site of the helicase nsp13 and the catalytic site of the RNA-dependent RNA polymerase nsp12, both components of the viral replication−transcription complex. We present the data on a public web portal (https://www.thesgc.org/SARSCoV2_pocketome/), where users can interactively navigate individual protein structures and view the genetic variability of drug-binding pockets in 3D.



INTRODUCTION

The coronavirus SARS-CoV-2 that emerged in late 2019 has so far caused over three million deaths worldwide. Whereas the recent approval of the first vaccines is expected to terminate the pandemic nature of the epidemic, it is believed that COVID-19 will not be eradicated, emphasizing the need for drugs.[1] Available vaccines may be less effective against novel and future SARS-CoV-2 variants, a serious global health threat. Additionally, in the years to come, novel coronavirus outbreaks are bound to emerge, as did SARS-CoV-1 in 2003, the MERS coronavirus in 2012, and SARS-CoV-2 in 2019. To simultaneously develop anti-COVID-19 therapeutics and prepare for the next pandemic threat, SARS-CoV-2 drug discovery efforts should focus on the development of pan-coronavirus agents. A first strategy is to target host proteins that are essential for the replication of past coronaviruses and may therefore be essential for the replication of coronaviruses emerging in the future.[2] An alternative approach, and the focus of this work, is to identify drug-binding sites in the SARS-CoV-2 proteome that are conserved across coronaviruses. An approved anti-COVID-19 drug binding such a site could be rapidly deployed against future outbreaks of novel coronaviruses.

Recent reports have provided valuable insight into the druggability of SARS-CoV-2 proteins[3] on their variation in COVID-19 samples and into their divergence from SARS-CoV-1 and from a specific bat coronavirus.[4] The excellent accompanying COVID-3D web portal allows users to map mutations on SARS-CoV-2 protein structures. A powerful online interface for inspecting variations between the SARS-CoV-2 genome and other viruses was also developed,[5] but to our knowledge, no integrated analysis focusing on the genetic variability of drug-binding pockets across the SARS-CoV-2 proteome has been reported to date.

RESULTS AND DISCUSSION

We analyzed the structures of all SARS-CoV-2 protein domains represented in the Protein Data Bank (PDB). The proteins analyzed included nonstructural proteins (nsp) nsp1, nsp3, nsp5, nsp9, nsp12, nsp13, nsp14 (a SARS-CoV-1 structure was used that shared 100% sequence identity with SARS-CoV-2 at the active site), nsp15, nsp16; structural proteins E (transmembrane domain) and Spike; and additional factors ORF3a, ORF7a, ORF8, ORF9b. We used the PocketFinder function[6] in ICM (Molsoft, San Diego) to find potential druggable binding sites and assessed their druggability using SiteMap (Schrodinger, NY).[7] The druggability evaluated with SiteMap was in general agreement with a previous work using a different method[3] and varied widely from poorly druggable, such as the catalytic site of the endoribonuclease nsp15 (druggability score [Dscore] < 0.8),

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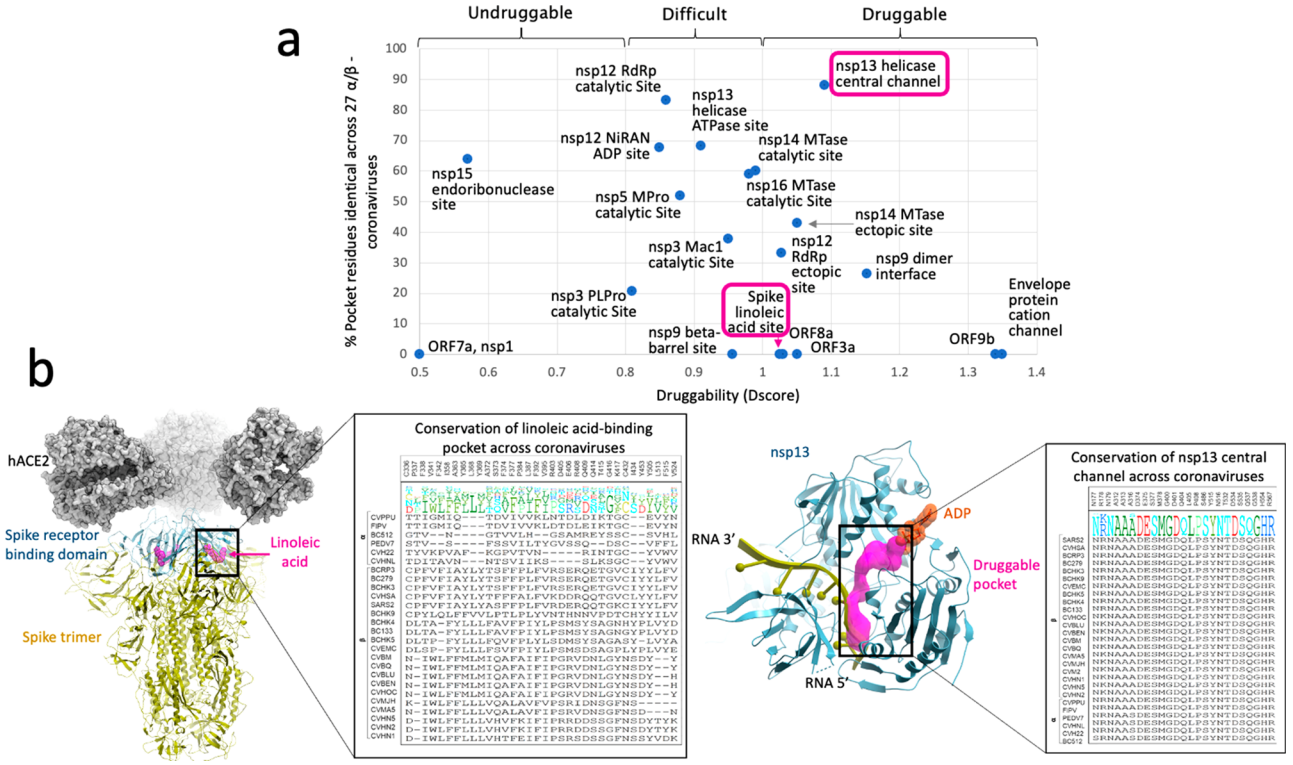

Figure 1. Druggability and conservation of drug-binding sites in SARS-CoV-2 proteins. (a) For all binding sites, druggability scores calculated with SiteMap[7] and the conservation of pocket-lining residues across α- and β-coronaviruses are represented in a scatterplot. (b) Structural details and multiple sequence alignments are shown for the linoleic-acid binding site of the protein Spike (left, PDB code 6ZB4), which is poorly conserved, and a channel overlapping the RNA binding site of nsp13 (right, PDB code 6ZSL with RNA from structure 2XZL), which is highly conserved. Both sites are highlighted in the scatterplot in panel a. The full names of all viral genera are provided in Supplementary Table S2.

to highly druggable, such as a functionally uncharacterized peptide-binding site on the nsp9 dimer (Dscore >1.0) (Figure 1a). The conformational variability of each site was also assessed by calculating the average B-factor across residues lining the pocket (Supplementary Table 1).

Because pathogenic coronaviruses responsible for mild or severe respiratory illness all belong to the genera alphacoronavirus (α-CoV) and betacoronavirus (β-CoV), we focused on these families to define the genetic variability of binding pockets found on the SARS-CoV-2 proteome.[8] Over 400 reviewed protein sequences of 58 α- and β-CoV were downloaded from UniProt (https://www.uniprot.org/). An automated sequence search retrieved homologues for most SARS-CoV-2 proteins, including all nsp proteins specified above, in 27 α- and β-CoV-comprising zoonotic entries (bat and bovine CoVs) and clinical and epidemic CoV isolates (SARS-CoV-1, MERS, HCoV-OC43, HCoV-HKU, HCoV-229E, HCoV-NL63). We performed multiple sequence alignments and determined the percent identity and the conservation of amino acids lining each binding pocket across all 27 coronaviruses where protein homologues were found (Figure 1a, Supplementary Figures 1 and 3, and Supplementary Table 2). Sequence variation was also evaluated across β-coronaviruses only, as SARS-CoV-1, MERS, and SARS-CoV-2 all belong to this group (Supplementary Figures 4 and 5). We find that some binding sites are highly conserved, whereas others are genetically unstable. For instance, binding of the essential fatty acid linoleic acid (LA) to the receptor binding domain of the SARS-CoV-2 Spike protein stabilizes the closed/locked state of Spike, inhibits Spike binding to ACE2, and synergizes with remdesivir to reduce SARS-CoV-2 replication, suggesting that drugs targeting this site may be of therapeutic interest.[9] Whereas the LA binding site is druggable (Dscore >1.0), we find that side chains lining LA are poorly conserved (0% conservation across 27 α- and β-coronaviruses), indicating that this site is not favorable for the discovery of pan-coronavirus inhibitors (Figure 1b). Conversely, a druggable channel partially overlapping with the RNA-binding site of the SARS-CcoV-2 helicase (nsp13) is highly conserved (>90% of residues lining the pocket are conserved across 27 α- and β-coronaviruses) (Figure 1b). Interestingly, nsp13 binds to the RNA-dependent RNA polymerase (nsp12) to functionally couple helicase and polymerase activities,[10] and we find that the catalytic site of nsp12 is also among the most conserved binding pockets in the SARS-CoV-2 proteome (>90% conservation across 27 α- and β-coronaviruses) (Figure 1a). These results support the notion of a strong selective pressure against genetic variability that may affect the function of the replication−transcription complex.

To further investigate genetic variability, we assessed the mutation of residues lining our collection of binding pockets across thousands of SARS-CoV-2 samples from COVID-19 patients and found some correlation with amino-acid conservation across α- and β-coronavirus genera (Supplementary Figure S3). For instance, the nsp13 druggable channel that is highly conserved across α- and β-coronaviruses is also the most conserved across SARS-CoV-2 samples: not a single residue lining this site was mutated across 15 000 samples. Conversely, the linoleic acid binding site of the Spike protein, which is poorly conserved across coronaviruses, is one of the

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J. Proteome Res. XXXX, XXX, XXX−XXX

most mutated across SARS-CoV-2 samples (38% residues mutated across 15 000 samples).

Our effort to systematically map the genetic variability of putative drug-binding sites onto 3D structures of the SARS-CoV-2 proteome reveals medicinal chemistry strategies for the development of broad-spectrum coronavirus inhibitors. The number of sequenced SARS-CoV-2 genomes from COVID-19 patient samples is continuously growing, and our analysis represents only a snapshot in time of the SARS-CoV-2 viral drift. Because this snapshot is based on over 15 000 sequences, we believe that it properly represents the relative mutational burden at each drug-binding site analyzed. In particular, we find that the RNA binding site of nsp13 and the catalytic site on nsp12 are not only the least mutated in SARS-CoV-2 but also the most conserved across coronaviruses, indicating that pharmacologically targeting the viral replication−transcription complex is a promising avenue for the discovery of pan-coronavirus drugs. In this regard, compounds such as EIDD-280, an oral antiviral targeting the catalytic site of nsp12 and currently in phase II−III clinical trials against COVID-19,[11] represent promising drug candidates for current and future coronavirus pandemic threats. Similarly, targeting the RNA binding site of nsp13 is a priority—though underexplored—avenue that may benefit from advances in the chemical inhibition of RNA-binding proteins[12] and from a recent crystallographic fragment screening effort that identified starting material for hit optimization.[13]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jproteome.1c00206.

Percent sequence identity and druggability of drug-binding sites in the SARS-CoV-2 proteome represented in the PDB (Figures S1−S5); mutation level of residues lining drug-binding sites found in SARS-CoV-2 proteins in the PDB across >15 000 samples from COVID-19 patients and across 27 α- and β- coronavirus genera (Figure S3); average B-factors at the analyzed binding sites (Table S1); conservation matrix of the SARS-CoV-2 proteome represented in the PDB across 27 α- and β-coronaviruses (Table S2); and detailed methods (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CoV, coronavirus; nsp, nonstructural protein; PDB, Protein Data Bank; Dscore, druggability score.

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