

overlaid with those of the target ligand. Flexible backbone sequence design was used to build the remainder of the sequence. Finally, low-energy, well-packed designs were validated by ab initio folding of sequences to establish designs that retained uncollapsed and preorganized binding sites in the absence of the bound target ligand. No subsequent downstream redesign was needed to enhance structural stability, function, or ligand-binding activity. This accomplishment represents an important step forward for de novo functional protein design.

To achieve the full potential of de novo protein design, simultaneous design of function is required. Unfolded proteins, or those with intended or accidental mutations, can be nonfunctional, whereas biology's successful and intended folds offer function. Given that the repertoire of biological function has evolved through select evolutionary pressures, designed proteins that achieve the same function are unlikely to offer substantial advantages, so new functionality beyond the scope of biology is the goal.

Recent developments, including the simultaneous design of protein structure and ligand-binding site by Polizzi and DeGrado, will provide exciting opportunities in sensing, light capture and storage, diagnostics,

“Ligand chemical-group locations relate to backbone coordinates..., so vdMs link directly to the protein fold.”

therapeutics, and catalysis, among others. The protein design community is now poised to design functional proteins that can begin to address some of the most pressing challenges facing society today, including ones in energy, health care, and sustainability. New protein design algorithms need to be made accessible to the nonexpert user, in a similar way to the protein design online computer games (7), if researchers with new creative functions in mind are to realize the full potential of protein design. ■

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CORONAVIRUS

A molecular trap against COVID-19

Structure-function studies reveal a new receptor decoy to block virus entry

By **Brandon J. DeKosky**

The cell surface peptidase angiotensin-converting enzyme 2 (ACE2) is the primary receptor for the spike (S) fusion protein that facilitates cell entry of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Numerous studies are evaluating therapeutic and preventive treatments that block S protein interactions with ACE2 molecules that are expressed on host cells. For example, the ACE2 binding site can be occluded by monoclonal antibodies, several of which are rapidly advancing in clinical trials. Several vaccines undergoing clinical development also induce antibody responses that block ACE2-S protein interactions. On page 1261 of this issue, Chan *et al.* (1) perform high-throughput mutagenesis and screening to reveal ACE2 mutations that enhance affinity for S protein, providing new insights into the ACE2-S protein interaction on which infection critically depends. The authors propose a strategy to apply engineered recombinant ACE2 variants as decoy receptors for coronavirus disease 2019 (COVID-19).

The dimeric ACE2 enzyme is a vasopeptidase expressed on the surface of epithelial cells in many tissues, including the lung, heart, blood vessels, kidneys, and gastrointestinal tract. It has a primary role in reducing blood pressure and inflammation as part of the renin-angiotensin-aldosterone system. ACE2 expression is closely associated with the tissue tropism of SARS-CoV-2 infection (2). The trimeric S protein comprises two subunits, S1 and S2. The S1 subunit contains a receptor binding domain (RBD), which binds to ACE2.

In addition to ACE2 binding, a protease cleavage of S protein is required for cell entry to allow S1 to release and reveal the hydrophobic cell fusion peptide of the S2 subunit (3). The cleavage between S1 and S2 can be accomplished by several different proteases, including transmembrane protease serine 2 (TMPRSS2), which is expressed in select

tissues, and cathepsin L, which becomes activated in the low-pH endosomal environment (4, 5). The role of ACE2 in facilitating S1 shedding remains to be determined (6). Recent data show that S protein undergoes a conformational rearrangement at endosomal pH that modifies S trimer interactions and rotates the RBD from the “up” to the “down” conformation, which also influences ACE2 binding affinity (7). The predominant reliance of SARS-CoV-2 on ACE2 for cell entry has led to a focus on the development of new methods to disrupt ACE2 binding to S protein as potential COVID-19 medical interventions.

Chan *et al.* performed protein engineering studies to understand SARS-CoV-2 RBD and ACE2 specificity characteristics and to engineer high-affinity ACE2 variants that could serve as a receptor decoy and compete with native ACE2 for binding to the RBD on SARS-CoV-2. They demonstrate that a number of residues in ACE2 can be further optimized to enhance ACE2 affinity for soluble RBD binding. The mutations providing enhanced or decreased affinity give important insights about ACE2-RBD interactions. One key finding was that disrupting the Asn⁹⁰ glycosylation motif in ACE2 enhanced the RBD binding affinity, by about twofold for the Thr⁹²→Gln ACE2 variant. Because glycosylation is a heterogeneous posttranslational modification that varies within cells and between cell types, this finding implies a potential for nonglycosylated ACE2 molecules to be more permissive to infection than fully glycosylated ACE2. Chan *et al.* identified several other mutations at the ACE2-RBD interface that reveal more structural features of the ACE2-RBD complex, including for ACE2 residues 27 to 31 at the binding interface, and several other mutations that suggest the influence of longer-range protein folding interactions.

As in Chan *et al.*, comprehensive mutagenesis and functional screening are being used extensively to interrogate virus-cell and antibody-virus interactions related to SARS-CoV-2. Chan *et al.* screened cellular receptor variants using mammalian cells, whereas another recent study performed a similar analysis of the RBD domain of S protein with yeast display, revealing structural constraints and affinity landscapes on the viral

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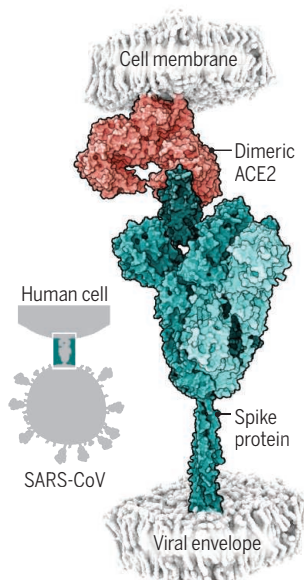
side of the ACE2-RBD interaction (8). Mutagenesis and screening of antibody proteins have been used for decades to elucidate antibody structure-function relationships and are now being used to improve antibody drug discovery against SARS-CoV-2. These mutagenesis and screening studies are accelerated by next-generation sequencing and provide rapid, high-throughput data on viral fusion interactions, along with opportunities for protein engineering and therapeutic discovery of antiviral vaccines and biologics.

Chan *et al.* outline a strategy to use an enhanced-affinity engineered ACE2 variant as a receptor decoy to block S protein on SARS-CoV-2. One engineered ACE2 variant, sACE2_{v2.4}, showed ~10-fold enhanced potency for preventing infection in vitro (i.e., neutralizing the virus) compared with wild-type ACE2. sACE2_{v2.4} showed a median inhibitory concentration (IC₅₀) neutralization potency against SARS-CoV-2 that was subnanomolar. The potency of dimeric sACE2_{v2.4} compared favorably to neutralizing monoclonal antibody potencies—only a small subset of monoclonal antibodies also exhibit subnanomolar IC₅₀ values. sACE2_{v2.4} also neutralized SARS-CoV (which causes SARS), suggesting that the engineered ACE2 mutations affect conserved interactions among the two related coronaviruses that both use ACE2 as a cell-entry receptor. Other receptor decoys have been pursued as antivirals, including against HIV (based on the CD4 host cell receptor) (9) and human rhinoviruses [based on the host cell receptor intercellular adhesion molecule (ICAM)] (10). Receptor decoys have not yet led to a clinically approved antiviral medication, but some have been demonstrated to be safe in human trials and showed efficacy in reducing viral titers and symptom severity in a controlled prevention and challenge study of the common cold (11).

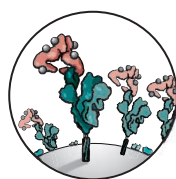
Engineered ACE2 receptor decoys are another addition to a panoply of exciting new strategies to block COVID-19 by disrupting ACE2-S protein interactions, where major parallel efforts are under way (see the figure). RBD-focused vaccines elicit antibodies that neutralize SARS-CoV-2 by directly blocking ACE2 binding. Recombinant S protein vaccines, whole-virus inactivated vaccines, and live-attenuated vaccines also elicit antibodies that interrupt binding to ACE2, in addition to other viral epitope targets. SARS-CoV-2

Blocking infection

There are multiple approaches to prevent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike (S) protein from binding to its cell entry receptor, angiotensin-converting enzyme 2 (ACE2).

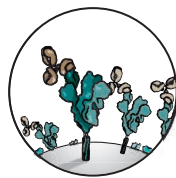


The SARS-CoV S protein-ACE2 structure is shown because the equivalent structure for SARS-CoV-2 is not available, but they are predicted to be similar.



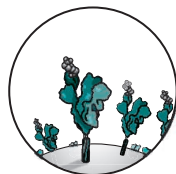
Engineered ACE2 decoys

ACE2 mutants are engineered to bind S protein more tightly than native ACE2, which inhibits SARS-CoV-2 infection in vitro.



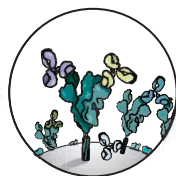
Monoclonal antibodies

S protein-specific antibodies block S protein and prevent viral fusion.



Small molecules or peptides

Molecules inhibit ACE2 binding by S protein.



Vaccine-elicited antibodies or convalescent serum

Naturally produced antibodies are specific to S protein and block ACE2 binding sites.

vaccine delivery strategies vary broadly, and numerous vaccines are advancing rapidly through clinical trials. Several small molecules have also been identified to block ACE2, including lectins and synthetic peptides derived from ACE2 (12). Small molecules can have key advantages in cost, production, stability, distribution, and administration compared with biologics. However, the less precise mechanisms of action and thus the potential for side effects increase clinical risk of small-molecule ACE2-S protein binding inhibitors. Monoclonal antibodies and vaccines possess a very different risk profile than small-molecule drugs. Perhaps the highest risk is antibody-dependent enhancement (ADE), where antibody Fc interactions can promote inflammation in respiratory mucosa that causes immunopathology. ADE is often associated with poorly neutralizing antibodies and has been reported for other respiratory vaccines and in prior studies of Middle East respiratory syndrome (MERS) and SARS (13). Fortunately, convalescent plasma (which contains antibodies from recovered COVID-19 patients) therapy has revealed no substantial ADE burdens (14), and potentially neutralizing monoclonal antibodies are less likely to cause ADE.

As a new biologic therapy, soluble ACE2-based receptor decoys have the advantage of no associated ADE risks, and recombinant ACE2 has an established clinical

safety record for treating pulmonary arterial hypertension and acute respiratory distress (clinical trials NCT01597635 and NCT03177603). The major disadvantage for soluble ACE2 as a COVID-19 preventive may be its relatively short half-life [~10 hours in prior studies (15)], suggesting that it may be best suited for treating COVID-19. The half-life could be increased for prevention indications by fusing them to an immunoglobulin G Fc domain. In addition to viral neutralization, therapeutic ACE2 could alleviate COVID-19 symptoms by decreasing inflammation and fluid accumulation in lung tissue, making engineered ACE2 biologics a promising approach to treat COVID-19 that may synergize with other treatment modalities.

New COVID-19 treatments and preventions are advancing rapidly, with numerous approaches to disrupt ACE2-mediated viral entry. Clinical trial results for the first generation of therapies will likely be announced at an accelerating pace toward the end of 2020, and additional structural information regarding ACE2 interactions with RBD and clarification of viral cell-fusion mechanisms will inspire new drugs to disrupt SARS-CoV-2 infection. Several challenges remain, including the logistical burdens of deploying medical interventions to blunt the spread of SARS-CoV-2. Methods of blocking ACE2-dependent viral entry that build on the growing understanding of ACE2 interactions may provide some of the strategies needed to suppress SARS-CoV-2, and other future coronaviruses. ■

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