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ACE2, the Receptor that Enables the Infection by SARS-CoV-2: Biochemistry, Structure, Allostery and Evaluation of the Potential Development of ACE2 Modulators

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Dedicated to the victims of the COVID-19 pandemic
Abstract  
Angiotensin Converting Enzyme 2 (ACE2) is the human receptor that interacts with the Spike protein of coronaviruses, including the one that produced the 2020 coronavirus pandemic (COVID-19). Thus, ACE2 is a potential target for drugs that disrupt the interaction of human cells with SARS-CoV-2 to abolish infection. There is also interest on drugs that inhibit or activate ACE2, i.e. for cardiovascular disorders or colitis. Compounds binding at alternative sites could allosterically affect the interaction with Spike protein. We here review biochemical, chemical biology and structural information on ACE2, including the recent cryoEM structures of full length ACE2. We conclude that ACE2 is very dynamic and that allosteric drugs may be developed to target ACE2. At the time of the 2020 pandemic, we suggest that available ACE2 inhibitors or activators in advanced development should be tested for their ability to allosterically displace the interaction between ACE2 and the Spike protein.

1. General Introduction

The Angiotensin Converting Enzyme 2 (ACE2), first described with a role in the renin-angiotensin system [1], is now considered a regulator of cardiovascular physiology, dietary amino acid homeostasis, innate immunity and gut microbial ecology. It is also the receptor of the severe acute respiratory syndrome coronaviruses (SARS-CoVs), including SARS-CoV-2, responsible for the 2020 pandemic. A number of excellent reviews focusing on different aspects of ACE2 function and relevance in disease have been published [2-8]. An earlier review had a focus on biochemistry, including details on ACE2 glycosylation, substrate specificity, requirement of pH and Cl⁻ for activity [9]. Here we review the structural and biochemical information on ACE2, analyse its dynamics and allosteric properties, and assess the possibility of identifying small compounds that allosterically disrupt the interaction with SARS-CoV-2.

1.1. ACE2

ACE2 is a transmembrane protein with an extracellular carboxypeptidase domain, located at the cell membrane in a variety of epithelial cells, including lung and airways, olfactory system, heart, kidneys, liver, pancreas and intestine [2-8,9]. ACE1 and ACE2 are coded by different genes and share a 40% amino acid sequence identity in the catalytic domain. While ACE1 catalyses the formation of Angiotensin II (Angiotensin 1-8; DRVYIHPF) leading to vasoconstriction, increased blood pressure, cardiac hypertrophy and inflammation among other effects, ACE2 processes Angiotensin I to Angiotensin 1-7 (DRVYIHP) and Angiotensin I to Angiotensin 1-9 (DRVYIHPFH), both enhancing vasodilatation and reducing blood pressure (Fig. 1). Thus, ACE2 is protective in multiple cardiovascular diseases, such as hypertension and heart failure [10-11]. The difference at the active sites between ACE1 and ACE2 is such that ACE1 inhibitors (i.e. enalapril, lisinopril, captopril) do not cross react with ACE2. ACE2 inhibitors have been developed, including MLN-4760 (also termed GL1001), DX-600, and 416F2 [12-14]. Inhibition of ACE2 with MLN-4760 showed beneficial effects on a model of colitis in mice [15] and entered clinical trials for the treatment of Ulcerative Colitis (www.clinicaltrials.gov).

Figure 1. Simplified scheme on the role of ACE2 in the renin-angiotensin system. The cleavage of Angiotensinogen by the enzyme Renin results in the decapeptide Angiotensin I (1-10). Angiotensin Converting Enzyme 1 (ACE1) cleaves Angiotensin I to Angiotensin II (1-8). Angiotensin II is a potent vasoconstrictor that binds to the type 1 Angiotensin II Receptor (AT1R) to set off actions that result in higher blood pressure and inflammation. Angiotensin Converting Enzyme 2 (ACE2) cleaves Angiotensin II to produce Angiotensin 1-7 which binds to Mas Receptor (MasR) producing vasodilatation and other cardiotrophic actions. ACE2 is cleaved by ADAM17 which releases the active ACE2 protease catalytic domain to the circulation.

ACE2 is cleaved at the cell membrane by the ADAM17 protease (tumour necrosis alpha convertase, TACE) [16], and by other proteases such as TPRRSS2, HAT and hepasin [17-18]. The cleavage by ADAM17, in a process termed shedding, releases catalytically active soluble forms of ACE2 into the circulation, with a still unclear physiological function [19-20]. Recombinant human ACE2 18-740 (rhACE2; i.e. GSK2586881/APN01) is being tested in clinical trials for diverse disorders including lung injury and pulmonary arterial hypertension [21]. GSK2586881/APN01, and also B38-CAP, a bacterial-derived carboxypeptidase, which cleaves both Ang I and Ang II to Ang 1–7 [22], are also in clinical trials for the treatment of SARS-CoV-2 infections.

ACE2 exerts indirect physiological functions on intestinal amino acid homeostasis, such as expression of antimicrobial peptides, and on the gut microbiome, by stabilizing the amino acid transporter B’AT1 with a chaperone-like mechanism [5,23]. In animal models the expression of ACE2 is upregulated by inhibitors of ACE1 and AT-R blockers, but the results have not
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been replicated in humans [24]. ACE2 expression is enhanced by interferon IFNs in human airway epithelial cells [25].

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Dr. Alejandro E. Leroux is a biochemist and PhD (University of Buenos Aires). After a postdoctoral research in Heidelberg (Germany), he returned in 2016 to Buenos Aires (IBioBA-CONICET). His research interests include protein kinase regulation by allosteric mechanisms and the characterization of molecular mechanisms leading to protein aggregation.

Prof. Dr. Stefan Zeuzem did his medical training at Goethe-University Medical School, Frankfurt Germany, where he is presently the Dean and heads the Clinic of Internal Medicine I. with responsibility over gastroenterology, hepatology, endocrinology, diabetology and pulmonology. SZ has a deep focus on anti-viral clinical research where he played a major role in the development of today’s anti-viral drugs against hepatitis C. SZ has received multiple recognitions and awards for those anti-viral developments.

Prof. Dr. Dr. Albrecht Piiper, originating from Göttingen, has received his MD in surgery and specialization in cardiology at the Frankfurt University Hospital. He is Head of the Clinic of Internal Medicine I, with a focus on research, innovation and outcome driven medical care. Over the last two decades, rational allosteric drugs have been developed to the G protein-coupled receptor family [46] including Maraviroc and Cincacalacet. Allosteric drugs such as Nevirapine [46] and Sofosbuvir [47] have also provided treatment options for human immunodeficiency virus and human hepatitis C virus infections. When compared to drugs binding at orthosteric sites, allosteric inhibitors can add the benefit of higher selectivity. Interestingly, as could be the case for ACE2, drugs directed to the orthosteric/active sites of enzymes can also produce effects on distant sites and allosterically enhance or disrupt interactions [48].

Studies on allosteric drug development to allosteric proteins show that the allosteric effect is often not visible from the analysis of crystal structures. Molecular dynamic studies can predict if a small compound binding at a first site can induce rigidity or mobility at a second distant site; however, rigidity or mobility at a particular site does not directly relate to increase or decreased binding of interacting proteins [49].

Specific studies on ACE2 dynamics and allostery from biochemical, chemical biology and structural work published over the years.

2. Deduced dynamics and allostery from biochemical and structural studies on ACE2

In the following paragraphs we summarize biochemical and structural knowledge on ACE2 that can inform about the possible existence of allostery and participation in an allosteric network.

2.1. Biochemical studies

ACE2 consists of a catalytic domain (Protease catalytic Domain, PD) and a Collectrin-like domain (CLD) that includes a neck
domain, a single transmembrane sequence and a cytoplasmic 43 amino acid tail (Fig. 2A,B).
The enzymatic activity of the PD can be inhibited by compounds or polypeptides binding to the substrate-binding site, i.e. MLN-4760. MLN-4760 does not affect the interaction of ACE2 with the Spike protein of the SARS-CoV-1 in immunoprecipitation experiments nor does it affect the coronavirus infection of cells in culture. MLN-4760’s potency is improved by increased chloride concentrations and mutagenesis analysis showed that Arg169 and Arg514 were critical to mediate the chloride-dependent increase in the potency of MLN-4760. Interestingly, Arg169 of ACE2 is approximately at a 16 Å distance from MLN-4760 binding site and so is unlikely to directly affect inhibitor binding, but rather to allosterically affect the active site. Supporting this hypothesis, chloride was also shown to modulate substrate selectivity of ACE2.

Is the ACE2 active site linked to other distant allosteric sites? While mutations of ACE2 at the active site render ACE2 inactive, the mutant protein still interacts with Spike protein and mediates infection, indicating that the catalytic activity of ACE2 is not required for SARS-CoV-1 interaction and infection. The effect of Spike protein S1 domain on ACE2 enzymatic activity was tested using purified recombinant ACE2 and a peptide substrate that fluoresces upon cleavage (Mca-YVADAPK(Dnp)-OH substrate). Li et al. found that the Spike protein does not affect the in vitro activity of ACE2—although the data are not shown in the manuscript. This result provides evidence that at least some interactions at the Spike-binding site may not affect the catalytic activity of ACE2. The finding suggests that if there was an endogenous protein binding to the Spike-binding site of ACE2, or a treatment with exogenous Receptor Binding Domain (RBD) from the Spike protein itself, it could block the interaction of human cells with SARS-CoVs without affecting ACE2 catalytic activity and its physiological function in the regulation of the renin-angiotensin system.

2.2. Structural and structure-based studies on the protease catalytic domain (PD)

There are over 20 crystal and cryoEM structures of the PD deposited in the Protein Data Bank. Most structures of the isolated PD show the catalytic domain with the active site in an “open” conformation (i.e. PDB 1R4L, 6M17, 6M18, 6LZG). A closed conformation is observed only in complex with MLN-4760 (PDB 1R42) [55] (Fig. 2C-D). There are also structures in an “intermediate” conformation, all of them in complex with the RBD of Spike from SARS-CoV-1 or a chimera of SARS-CoV-1 and 2 (i.e. PDB 6ACK, 2AJF, 6WV1). Open and closed conformations vary at the active site and at the “back”, along the hinge region (shown as schemes in Figure 2C).

Figure 2. Structure and Conformations of ACE2. (A) Scheme of motifs and domains of ACE2. ACE2 has an N-terminal Protease Catalytic Domain PD (blue) and a C-terminal Collectrin-like domain CLD (cyan). The first 17 amino acids correspond to the signal peptide that is cleaved during the maturation of the protein (not shown). The CLD consists of an extracellular Neck Domain, a linker, a single transmembrane (TM) helix and an intracellular tail of 43 amino acids. The cleavage site by proteases that release soluble ACE2 to the circulation are indicated. (B) Structure of full length ACE2 (tight-dimer) in complex with B’AT1 (PDB: 6M17). ACE2 is represented as cylindrical helices and loops with surface, while the surface of B’AT1 is presented in grey. For simplification, the RBDs present in this structure are not shown. The monomers of ACE2 are coloured in blue and pink shades (following the colours of A). The different regions of ACE2 and the 4 key regulatory sites (1. active site; 2. hinge; 3. Claw-like or Spike (RBD)-binding site; 4. PD dimerization interface) are indicated. (C) Schematic representation of the open-close hinge movement of the PD. The active site of the PD can adopt an open, intermediate (not shown) or closed conformation. The hinge pocket is disassembled in the closed structure. (D) MLN-4760 binds at the active site, stabilizes the closed structure and does not affect the interaction with Spike. (E) Small compounds designed to bind at the hinge region, i.e. diminazene, increases the activity of ACE2 (F).
Small compound designed to bind at the active site in the closed structure of PD (NAAE) displaces interaction with Spike protein. (G) Schematic representation of the structure of full length ACE2 dimers in two conformations identified by cryoEM in complex with B'AT1. In the absence of the Spike protein RBD, the two conformations are found in a 3:1 proportion. The tight-dimer (left) is a scheme representing the structure shown in B. In both dimer conformations the Neck domains form tight interactions. In the loose-dimer (right), the PDs rotate with respect to the Neck Domain and the PD-PD interaction is lost. In the presence of Spike protein RBD, only the tight-dimer ACE2 structure is present. In the loose-dimer the conformation of the Spike protein binding site in the PD appears modified (detailed in Figure 3). (H) Chemical structures: 1; XNT; 2; MLN-4760; 3; resorcinolnaphthalein; 4; NAAE; 5; diminazene. The mechanisms of action for XNT, resorcinolnaphthalein, NAAE and diminazene (E) and (F) are deduced from biochemical and not validated structurally.

Activators of ACE2 could be beneficial as drugs for the treatment of diabetic nephropathy, heart failure, or hypertension \[56\]. The structures of the isolated protease catalytic domain in open and closed conformations were used to screen *in silico* for compounds that bind at sites different from the active site. In a first study, Hernandez Prada et al. identified two small compounds (XNT and resorcinolnaphthalein) binding at the hinge region (Fig. 2B,C) that in an *in vitro* ACE2 activity assay (Mca-YVADAPK(Dnp)-OH substrate) activated ACE2 1.8 and 2.2 fold, respectively, with EC_{50} values (concentration to achieve 50% enhancement of activity) of approximately 20 µM \[57\]. Using the same approach, diverse FDA-approved drugs were identified as low µM binders and the compound diminazene was described to activate ACE2 (EC_{50} 8 µM), by modifying both Km and Vmax (Mca-YVADAPK(Dnp)-OH substrate) (Fig. 2E-H) \[58\]. Together, these studies suggest that there is an allosteric communication between the hinge region and the active site. There is also evidence for possible allosterity between the active site and the Spike protein binding site. Huentelman et al. performed an *in silico* screening based on the closed conformation of ACE2 to identify new compounds binding to the active site and described that the molecule NAAE (N-[2-aminoethyl]-1 aziridine-ethanamine) inhibits ACE2 activity (IC_{50} 50 µM) \[59\]. Treatment with this molecule inhibited SARS-CoV membrane fusion. The finding indicates an allosteric communication between the active site of ACE2 and its site of interaction with Spike protein from SARS-CoV-1 (shown schematically in Figure 2F), suggesting that drugs may be developed to bind at the active site and disrupt the interaction with the SARS-CoV-2 Spike protein \[59\].

2.3. Structural studies on full length ACE2

The Spike protein is a trimer. Interestingly, recent work shows that a monomeric form of the PD (ACE2 18-640) binds efficiently to the isolated RBD from Spike, but does not significantly bind full length Spike trimers, while the PD (18-640)-Fc dimer can bind full length Spike trimers with reduced on-rate but also reduced off-rate \[60\]. CryoEM studies with SARS-CoV virions show that it binds to three soluble ACE2-Fc molecules \[81\]. After interaction with ACE2, the Spike trimer undergoes conformational changes that promote membrane fusion. It is not yet known whether ACE2 must also undergo conformational changes to enable infection. The structure of full length ACE2 was recently elucidated by cryoEM in a complex with the amino acid transporter B'AT1 \[8\] (Fig. 2B). It shows that ACE2 is a dimer, as previously described biochemically \[61-62\]. Dimers provide additional possibilities for cooperative-allosteric effects, although these have not been described in ACE2. In the full-length solved structure, B'AT1 supports the formation of dimers by stabilizing the Neck Domain and the transmembrane helix of ACE2. This is in agreement with previous work showing that ACE2 constructs comprising the extracellular regions, Neck Domain and PD, are dimers. Dimers of ACE2 comprising PD fused to the Fc domain of antibodies, have also been employed in research \[61-62\].

The cryoEM solved structure of full length ACE2 revealed the existence of two types of dimers: the authors describe them as “closed-dimer” and “open-dimer” \[8\]. To avoid confusion with the “open” and “closed” conformations of the PD, we will refer to them as tight-dimer and loose dimer, respectively (shown schematically in Figure 2G). In the tight-dimer conformation the dimerization interface consists of a strong interaction between the CLD Neck domain and a second interaction between the two PDs. Interestingly, when the authors investigated by cryoEM the structure of full length ACE2 in the presence of the RBD, they identified only the ACE2 tight-dimer in complex with RBD (in the presence of 10 mM leucine). In the loose-dimer conformation, there is a rotation between the CLD and the PD (Fig. 2G, 3A), which breaks the dimer interaction between the PDs and separates both domains about 25 Å. As a result, the PDs do not interact, while the dimer still remains stable, mediated by the interface within the Neck domain. More notably, the cryoEM structure of the full length ACE2 reveals that the PD can be stabilized in a new structural “twisted” conformation (Fig. 2G and Fig. 3B). In this new conformation, the PD-claw-like surface shows changes which include the shifting of residues involved in the interaction with the RBD \[8, 63\], most notably at the α1 helix of ACE2, the main point of interaction with the viral protein (Fig. 2G and Fig. 3C).

3. Analysis on ACE2 dynamics and allostery

The structural information of ACE2 obtained by crystallography and cryoEM provide snapshots depicting conformations that ACE2 can stably achieve. Taken together with the biochemical studies, we propose a cartoon model of ACE2 with various key sites that appear dynamic (Fig. 2C-F). We identify four key sites on the PD, the carboxypeptidase active site (site 1, Fig. 2B,C), the hinge pocket (at the back of the active site (site 2, Fig. 3B,C) and the claw-like surface that interacts with the RBD within the S1 domain of the viral Spike protein (site 3, Fig. 2B,C). In addition, based on the cryoEM full length structure, we must also consider the existence of a dimerization interface/site (site 4, Fig. 2B, F). How are these sites related to each other? The proteolytic domain can be observed in “open”, “closed” and “intermediate” conformations (Fig. 2C). These conformations refer to the “opening” of the active site, where the hinge region participates in the movement. The only structure in “closed” conformation corresponds to the crystal bound to the ACE2 inhibitor MLN-4760 (Fig. 2D). The finding that compounds binding at the hinge region, site 2, can modulate the protease activity, site 1, provides evidence that the two sites are allosterically connected (Fig. 2E). While MLN-4760 binding to the active site does not affect the binding of the Spike protein to ACE2 (Fig. 2F), NAAE designed to bind at the active site in the closed conformation, disrupted interaction with the Spike protein. Therefore, we must also consider that a subset of compounds binding at ACE2 active site (site 1) could affect site 3 and enhance or inhibit interaction with the Spike protein. The finding of the twisted conformation of the PD in the full-length loose-dimer shows a novel stable conformation of the PD (Fig. 2G). In addition, the
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A twisted conformation has a modified claw-like surface, which, if stabilized, could impair efficient binding to RBD from the Spike protein at the claw-like site (site 3) and inhibit interaction with SARS-CoVs.

It called our attention that the association of the cytoplasmic tail of ACE2 with a ubiquitous calcium-binding protein, calmodulin, reduces the cleavage and release of its extracellular peptidase domain [64-65]. One simple explanation could be that the interaction at the cytoplasmic domain induces a direct stabilizing effect on the extracellular region of ACE2, avoiding the exposure of the cleavage site or inhibiting the interaction with the protease. Although other mechanisms could also explain the finding, it is tempting to speculate that there is an allosteric communication between the cytoplasmic tail of ACE2 and the extracellular domain that could physiologically signal in both directions. Independently of the mechanism, pharmacologically affecting the calmodulin binding to the intracellular tail would be expected to modulate the stability of the extracellular domain.

Another hint of allostery between distinct sites on ACE2 comes from the apparent contradiction that while ACE2 inhibitors appear to be beneficial for colitis, the knock-out of ACE2 produces colitis [8]. ACE2 ameliorates colitis indirectly by stabilizing the neutral amino acid transporter B°AT1 by a mechanism that does not require ACE2 catalytic activity [66]. One possibility to explain this contradiction is that the ACE2 inhibitors binding at the active site, like MLN-4760, could stabilize ACE2, which will also stabilize B°AT1 allosterically. Since B°AT1 interacts with the Neck domain and the transmembrane helix of ACE2, a direct effect by MLN-4760 could imply an allostERIC communication between the active site of the PD and the CLD of ACE2. Following on the known interaction between B°AT1 and ACE2 it was also recently suggested that the B°AT1 inhibitor Nimesulide, approved drug in some countries, could potentially affect the interaction with the Spike protein, allosterically [67].

3.1. The strengths and limitations of the analysis

The main limitation is that the studies on ACE2 were not designed to investigate its dynamics and allostery. However, a strength is that there is consistent information suggesting that ACE2 is dynamic and possibly populated in equilibrium between different conformations depicted schematically in Figure 2. The in vitro biochemical characterization of small molecule “activators” comprises an important experimental evidence of allostery. However, Haber et al. indicated that, in their hands, the claimed small molecule “activators” did not affect the in vitro activity of ACE2 (using Mca-APK-Dnp as a substrate) [68]. It is possible that the results of both groups of researchers may be correct, but that the conditions of the assay by Haber et al. did not reveal an increase in activity. There are different reasons for such discrepancy. For example, an allosteric activator can act by affecting the Km; in such case, the concentration of substrate used could mask the activating effect; the different substrates should be well characterized before a study for possible allostery, particularly for the characterization of allosteric compounds when the readout is the activity. At any rate, the compounds claimed to activate ACE2 in vitro did produce the desired effect in vivo. Noteworthy, except for MLN-4760, a confirmation that the experimental compounds indeed bind at the expected sites is missing.

We here broadly assume that the understandings obtained with SARS-CoV-1 would turn out to be indistinguishable from SARS-CoV-2. Although several important aspects have been found to be identical, the specific studies on the interactions of ACE2 with the Spike proteins may vary, since the RBD of the SARS-CoV-2 Spike protein has additional residues participating in the interaction and higher affinity to ACE2 [63]. Studies on ACE2 identified that it is phosphorylated at residue 680, and that this phosphorylation stabilizes the protein avoiding degradation [69]. It is not known if the phosphorylation could affect any of the characteristics of the protein described biochemically, by crystallography or by cryoEM. Also we should keep in mind that many studies on ACE2 have employed ACE2 PD-Fc dimers, which may not mimic the physiological dynamics of ACE2 dimers.

![Figure 3. The rotated and twisted conformation of the full length ACE2 loose-dimer. The images are obtained by alignment of the tight-dimer (blue) and loose-dimer (red). (A) Rotation of the PD in relation to the CLD. The rotation is shown upon alignment of the CLD. (B) The structure of the PD in the rotated-twisted loose-dimer. The rotated PD is modified at the RBD-binding site. The image is produced by alignment of PD. The top region – which interacts with the RBD of the Spike protein undergoes changes, particularly in the α1 helix. The zoom depicts the RBD-binding site of ACE2 upon aligning the last C-terminal portion of the α1 helix. In the loose-dimer some of the helix α1 residues that interact with the Spike protein RBD move about 4.5 Å (measuring from the Co). The table indicates the relative movement between the tight-dimer and loose-dimer of Co of relevant residues that interact with the Spike protein RBD.](image-url)
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4. Summary and Outlook

Allostery is a central widespread mechanism in all life forms, once defined as "the second secret of life". In summary, ACE2 appears as a highly dynamic protein, where allostery has been demonstrated to different degrees between the active site (site 1), the hinge region (site 2), the claw-like/Spike protein binding site (site 3), and the CI binding site. In addition, there are potential allosteric communications between the active site and the stability of the B\(^{AT1}\), and between the intracellular tail and the cleavage of the extracellular domain releasing PD to the circulation. The twisted catalytic domain of ACE2 observed in the full length cryoEM structure hints to the existence of a structural communication between parts of the full length protein and the claw-like/Spike binding site, which may be exploited by drugs to allosterically inhibit the interaction with Spike protein. From the drug discovery perspective, the dynamic features of ACE2 and the knowledge accumulated throughout the years support the possibility that ACE2 conformation and function could be modulated by allosteric drugs. On the one hand, we suggest that further studies should confirm the allosteric nature of ACE2, the role in physiology and the potential for exploiting the allosteric properties for drug discovery. On the other hand, the small compounds identified in chemical biology or drug discovery projects will help to define more clearly the allosteric features of ACE2 and the possible exploitation for therapies. The screening of a small library of compounds using an AlphaLISA-based interaction assay between ACE2 and the RBD domain of the Spike protein identified small compounds that displace the interaction \(^{[70]}\). Such kinds of assays can identify small compounds that bind to ACE2 with different mechanisms of action to displace the interaction with Spike. Notoriously, one validated "hit" compound that displaced the interaction in vitro was the enalapril (IC\(_{50}\) 7.5 \(\mu\)M), a prodrug approved for the treatment of hypertension that is converted by de-esterification to enaprilat \(^{[70]}\), which is a potent ACE1 inhibitor. Together with the discussions above, the finding further highlights the possibility that enalapril, at high concentrations used, may cross-react with ACE2, binding at the active site and allosterically displacing the interaction with the coronavirus Spike protein as depicted in Fig. 2F. The identified approved drugs are active in vitro at too high concentrations for use as anti-virals, but could help to pave the way for future anti-SARS-CoV-2 drugs.

Given that ACE2 appears as a highly dynamic protein with a complex allosteric network between key sites, the development of compounds interacting at one side should ideally be tested for their effects on the different distant sites. For example, an activator compound that enhances ACE2 catalytic activity could have an effect on its ability to affect the interaction with the Spike protein, the stability of B\(^{AT1}\), the ability to be cleaved by ADAM17 or the intracellular interaction with calmodulin. Still, a major question is whether high affinity small compounds and drugs will be able to bind distinct sites on ACE2 to modulate its activity and its interaction with SARS-CoVs. Additional remaining questions related to ACE2 dynamics abound. Does ACE2 dynamic equilibrium between conformations have a physiological function? Is there an endogenous ligand that binds to the Spike protein binding site on ACE2? Does the interaction of the Spike protein with ACE2 allosterically affect any other conformation-dependent function of ACE2, i.e. B\(^{AT1}\) stability, shedding, or intracellular signalling? Does SARS-CoVs infection require ACE2 conformational changes and dynamics? Do ACE2 polymorphisms \(^{[71]}\) affect ACE2 dynamics and infection by SARS-CoVs?

Finally, in relation to the current COVID-19 pandemic we encourage the evaluation of all available drugs and advanced compounds targeting ACE2 for their abilities to allosterically inhibit the interaction with the Spike protein and to inhibit the infectivity by SARS-CoV-2.

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ACE2 is a membrane carboxypeptidase with an enzymatic role in the Renin-Angiotensin system and a chaperone-like function on intestinal amino acid intake. Moreover, ACE2 is the cellular receptor that enables interaction and infection by SARS-CoV-2, producing COVID-19. We here review biochemical, chemical biology and structural studies published on ACE2 with a focus on ACE protein dynamics, allostery and potential for allosteric drug development.