

# Structural insights into allosteric regulation and conformational dynamics of ACE2 in catalysis and SARS-CoV-2 binding

P. Giastas<sup>1,2</sup>, O. Kupreienko<sup>1,2</sup>, N. Alevizopoulos<sup>3</sup>, M.A. Balachouti<sup>3</sup>, D. Georgiadis<sup>3</sup>, A. Argyriou<sup>4</sup>, G. Spyroulias<sup>4</sup>, M. Zouridakis<sup>2</sup>

<sup>1</sup>*Department of Biotechnology, Agricultural University of Athens, Athens 11855, Greece,* <sup>2</sup>*Hellenic Pasteur Institute, Athens 11521, Greece,* <sup>3</sup>*Department of Chemistry, National and Kapodistrian University of Athens, Athens 15771, Greece,* <sup>4</sup>*Department of Pharmacy, University of Patras, Patra, Greece*

*Email of communicating authors: petrosgiastas@aua.gr and mzouridakis@pasteur.gr*

Angiotensin-converting enzyme 2 (ACE2) is a pivotal enzyme in the renin-angiotensin system, catalyzing the conversion of angiotensin II (Ang II) to the vasoprotective peptide angiotensin 1-7, with therapeutic potential for cardiovascular, hypertensive, and inflammatory disorders. Additionally, ACE2 serves as the primary receptor for SARS-CoV-2, yet its catalytic function remains underexplored as a drug target [1,2]. Here, we present a comprehensive structural and biophysical study of ACE2, focusing on its allosteric regulation, conformational dynamics during catalysis, and the impact of these dynamics on SARS-CoV-2 receptor binding domain (RBD) interactions.

We expressed and purified the monomeric catalytic domain of ACE2, establishing a robust platform for high-resolution crystallographic and functional studies. To probe the enzyme's conformational landscape, we determined crystal structures of ACE2 in complex with the SARS-CoV-2 RBD (open conformation) and with two transition state analogues: a phosphinic Ang II analogue and a smaller tripeptide mimic (named DG4). Both later structures reveal a dramatic hinge-bending motion, where the open, substrate-receptive groove collapses into a concave, semi-enclosed cavity, likely representing the catalytically active conformation. These complementary structures provide a detailed view of substrate processing and conformational flexibility. Through targeted small-molecule screening, we identified a novel activator that enhances ACE2 enzymatic activity by binding a previously unreported allosteric pocket within the internal cavity, distinct from the active site. Crystallographic analysis, including soaking experiments in the closed-state Ang II analogue-bound ACE2, confirms that the activator does not compete with natural substrates, underscoring its physiological relevance. Preliminary mechanistic studies suggest the activator boosts catalytic turnover without altering substrate affinity, offering a promising lead for therapeutic development. To resolve debates regarding ACE2 conformational influence on SARS-CoV-2 binding, we employed isothermal titration calorimetry (ITC), measuring dissociation constants of 25.9 nM for the open conformation and 7.2 nM for the closed conformation (induced by the modulator DG4). This modest affinity difference indicates that the RBD-binding site on subdomain I is minimally affected by hinge-bending, challenging previous assumptions that the viral entry could be influenced by ACE2 active site binders. High-resolution gel filtration experiments further demonstrated that the ACE2-RBD complex remains stable in both conformations, with DG4 neither disrupting preformed complexes nor impairing RBD binding. These findings suggest that conformational modulation is unlikely to suppress SARS-CoV-2 infection, highlighting the robustness of the ACE2-RBD interaction. Complementary experiments investigated whether RBD binding modulates ACE2 catalytic activity, addressing its dual role in viral entry and renin-angiotensin system regulation. Structural analysis reveals that the RBD-binding site on subdomain I is distant from the active site, and the internal catalytic cavity remains accessible whether ACE2 is apo or RBD-bound. This spatial separation suggests that RBD binding does not sterically hinder substrate access or restrict the hinge-bending dynamics, critical for catalysis. Together, these experiments demonstrate that ACE2 conformational flexibility enables robust RBD engagement and sustained enzymatic activity, as the independent operation of the RBD-binding and catalytic sites ensures neither function compromises the other.

Future efforts will focus on detailed kinetic and thermodynamic characterization of the determined allosteric activator, alongside structure-guided optimization to enhance its potency for treating ACE2-related pathologies, such as hypertension and acute respiratory distress syndrome. This work integrates crystallographic, biophysical, and medicinal chemistry approaches to unlock ACE2 allosteric potential, offering new insights into enzymology, allostery, and possibly antiviral strategies.

[1] McKinney, C. A., Fattah, C., Loughrey, C. M. et al. *Clin. Sci.* 2014, 126, 815–827.

[2] Nicolau, L. A. D., Nolêto, I. R. S. G., Medeiros, J. V. R. *Expert Rev Clin Pharmacol* 2020, 13, 807–811.

*We acknowledge Instruct-ERIC, iNEXT-discovery and EMBL Hamburg for supporting synchrotron access at beamline P13, the General Secretariat for Research and Innovation under the action TAA TAEDR-0535850 “BrainPrecision”, NextGenerationEU, National Recovery and Resilience Plan Greece 2.0 and the Hellenic Foundation for Research and Innovation (HFRI) under the HFRI PhD Fellowship grant to O. Kupreienko (Fellowship Number: 20774)*