The Catalytic Mechanism of the SARS-Cov-2 Main Protease

Henrique S. Fernandes, Sérgio F. Sousa, and Nuno M.F.S.A. Cerqueira

UCIBIO@REQUIMTE, i4HB, BioSIM
Department of Biomedicine, Faculty of Medicine of the University of Porto, Portugal

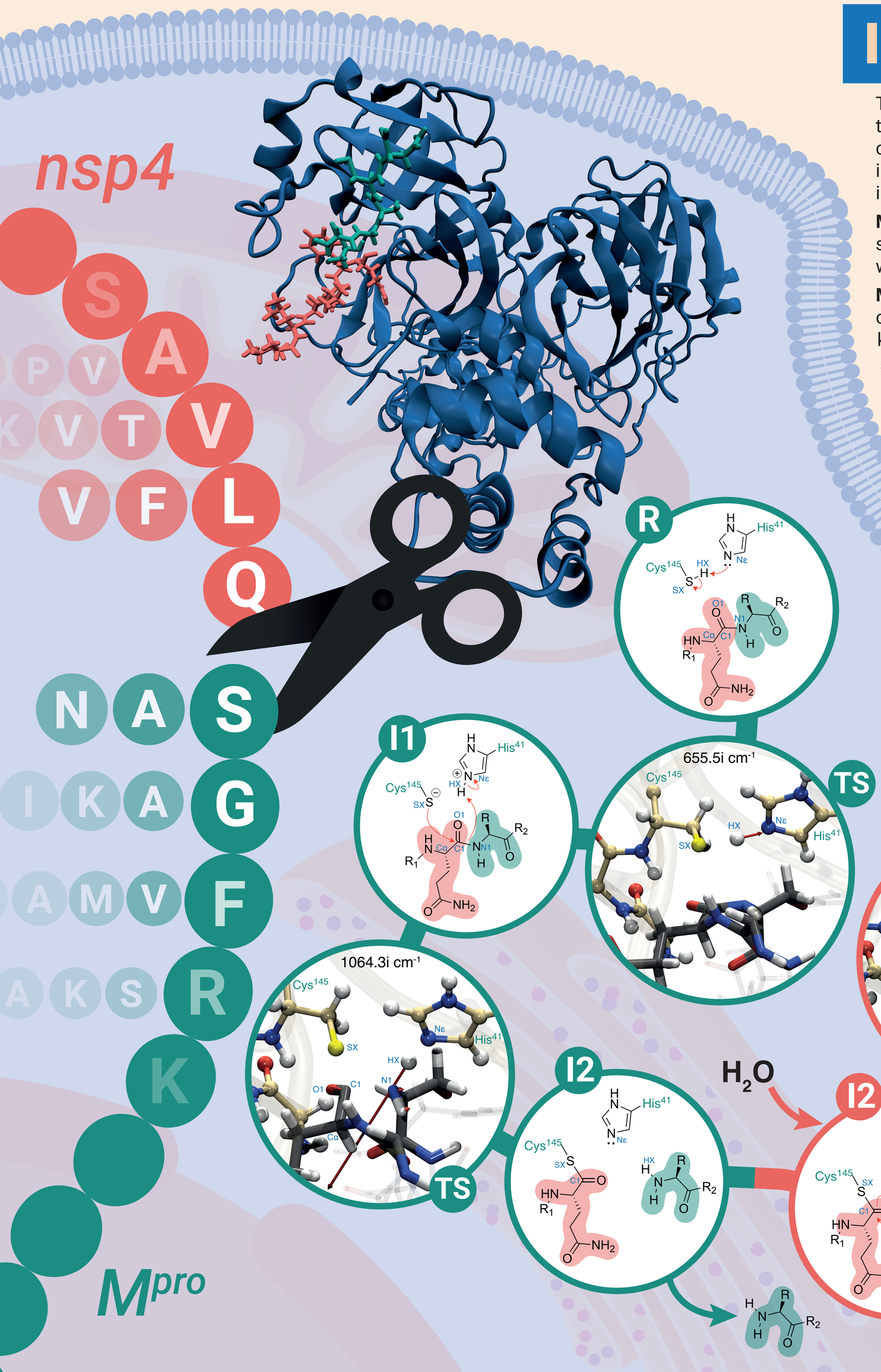
hfernandes@med.up.pt henriquefernandes.pt @Henrique_S_Fer

Biomolecular SIMulations Research Group
info@biosim.pt www.biosim.pt

Institute for Health and Bioeconomy

UCIBIO @REQUIMTE

FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO



INTRODUCTION

The main protease of SARS-CoV-2 (**M^{pro}**), is a key enzyme for the replication of the virus causing the COVID-19 pandemic. **M^{pro}** cleaves the viral polyprotein chain in **11 different regions**.[Nature 579, 265–269 (2020)] Therefore, the effective inhibiting **M^{pro}** activity can stall the viral replication cycle [Science 300, 1763–1767 (2003)], in a similar approach as the HIV-1 protease inhibitors [Wang, Y. et al. HIV 95 (2015)].

M^{pro} is a **homodimer** with two independent and highly solvent-exposed active sites, favoring the peptide substrate binding. Also, it ensures the availability of water molecules for the hydrolysis reaction.

M^{pro} catalyzes the cleavage of peptides with a well-established pattern. The cleavage always occurs between a **Gln** (P1) and a **Ser**, **Ala**, or **Asn** (P1'). The key **Gln** is always preceded by a bulky nonpolar residue: **Val**, **Leu**, or **Phe** (P2). According to the literature, the **P4-P1'** range comprises the most important residues for the recognition and anchoring of the peptide.[PNAS 113, 12997–13002 (2016)]

The reaction is catalyzed by a **Cys-His dyad**. The His41 residue enhances the nucleophilic character of Cys145 that covalently attaches the peptide substrate to the enzyme. This reaction intermediate is then hydrolyzed, and the product of the reaction is released to the active site.

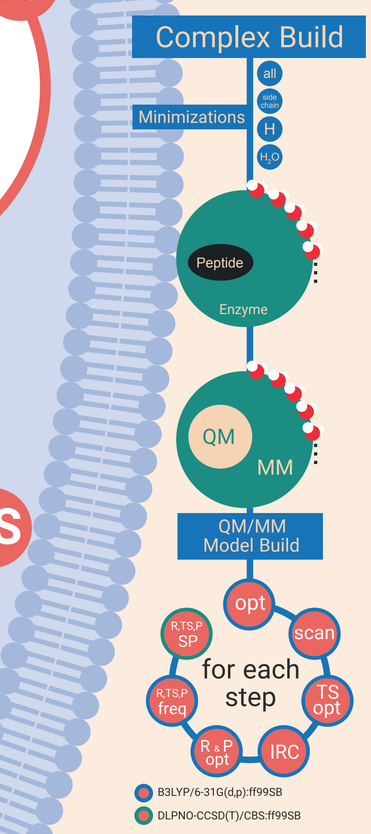
In this work, the catalytic mechanism of the SARS-CoV-2 **M^{pro}** was studied employing ONIOM QM/MM methodology (DLPNO-CCSD(T)/CBS//B3LYP/6-31G(d,p):AMBER).[Fernandes, H. S. et al. Mol Divers (2021)]

METHODOLOGY

This study was conducted based on the X-ray structure with the PDB code **6LU7**.[Nature 582, 289–293 (2020)] The protonation states were assigned using PROPKA 3 (pH 7), and TIP3P water molecules were used to solvate the system. Four sequential minimizations were conducted (ff14SB and 10 Å cut-off).

molUP[JCC 39, 1344–1353 (2018)] for VMD was used to prepare the QM/MM model with 69/72 atoms treated with QM. All geometry optimizations were performed with Gaussian09 and **B3LYP/6-31G(d,p):ff14SB** level of theory.

Single-point energy calculations were performed using ORCA 4.2.1. and **DLPNO-CCSD(T)/CBS**.



CONCLUSION

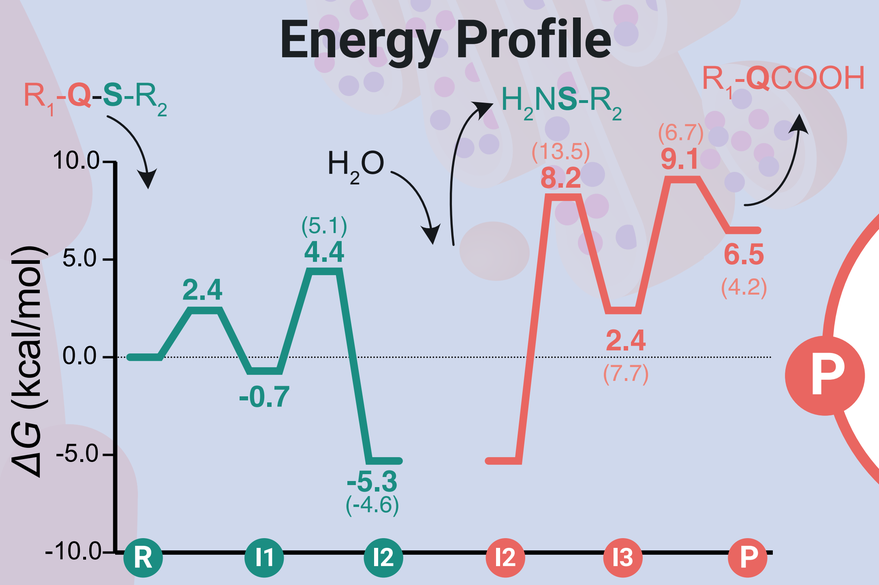
Full description of the **catalytic mechanism** with atomistic level of details.

Energy Profile computed with **DLPNO-CCSD(T) /CBS:ff14SB** level of theory.

Substrate's P2 residue interacts with His41, stabilizing its positive charge.

Substrate's **P1'** residue plays a key role in the stabilization of the ion pair intermediate.

Gly143, Ser144, and Cys145 are crucial for the stabilization of the substrate's oxo group.



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