SARS-CoV-2 M^{pro} inhibition by zinc ion: structural features and hints for drug design

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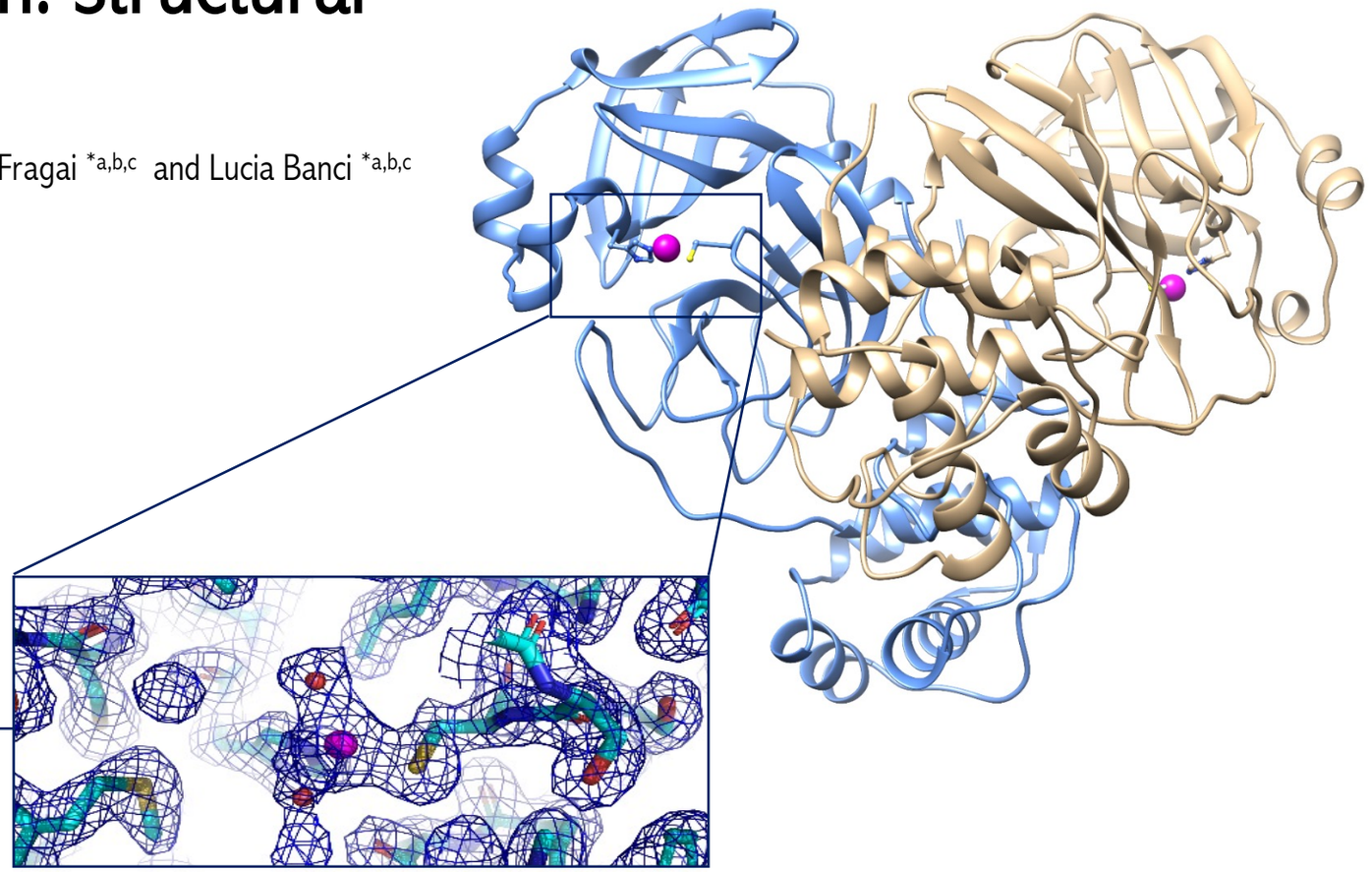
INTRODUCTION

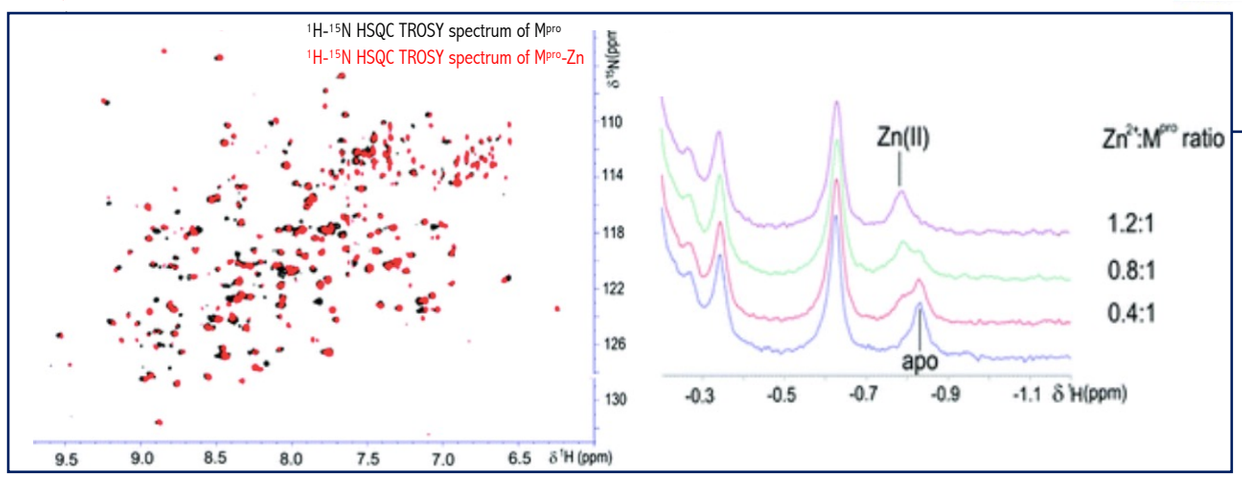
The SARS-CoV-2 main protease (SARS-CoV-2 M^{pro}) is a cysteine protease that hydrolyses viral polyproteins at several conserved sites. The enzyme represents one of the main drug-target candidates for covid-19 infection because it features a large and deep pocket at the active site and is crucial for viral replication[1]. Several inhibitors of SARS-CoV and SARS-CoV-2 M^{pro} have been designed to form a covalent bond between the thiol group of the catalytic cysteine and the inihibitor[2]. Here we report the X-ray structure of SARS-CoV-2 M^{pro} both in the apo form and in complex with an isolated zinc ion, and an extensive biophysical analysis of the metal–protein interaction properties in solution.

Crystal structure of SARS-CoV-2 M^{pro} bound to Zn^{2+}

(1.8 Å resolution PDB code: 7NWX)

Zn^{2+} is coordinated by the sulphur atom of Cys145, the Nε atom of the imidazole ring of His41, a well-defined water molecule and a more labile one shuttling between two positions, thus completing a tetrahedral geometry.



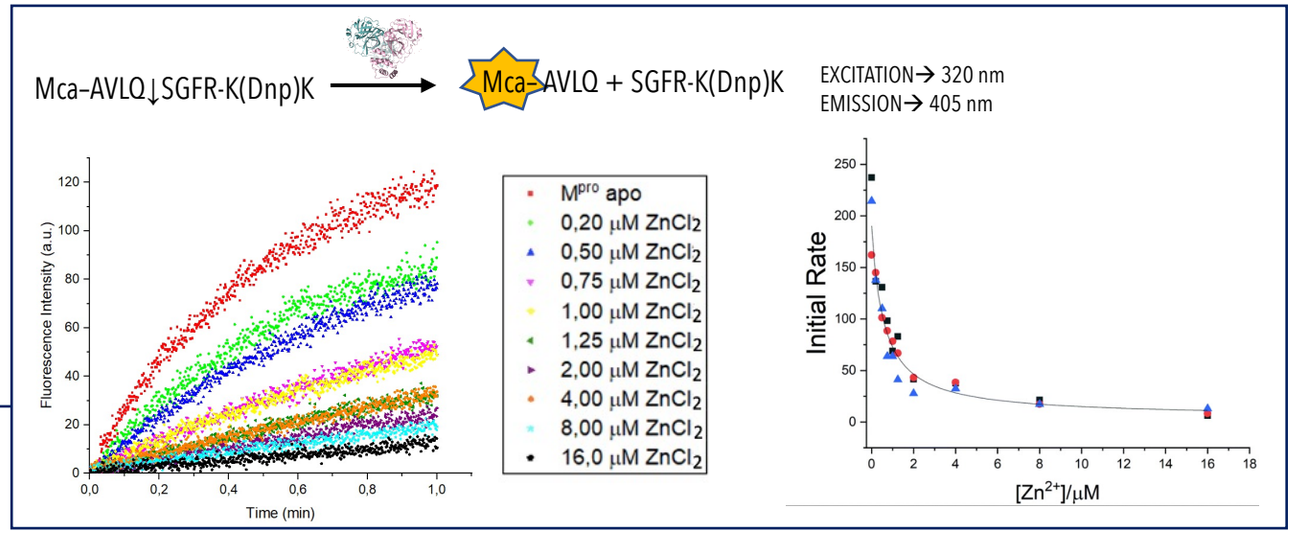


In solution interaction of SARS-CoV-2 M^{pro} with Zn^{2+} investigated by NMR

^{15}N isotopically enriched protein samples were titrated with Zn^{2+} and monitored using 1D ^{1}H and 2D ^{1}H–^{15}N TROSY HSQC NMR spectroscopy. Spectral changes were observed that indicate an intermediate-to-slow regime on the NMR time scale between the free and bound forms

Inhibition of M^{pro} proteolytic activity by Zn^{2+} studied by fluorescence assay

A fluorimetric assay was carried out by monitoring the fluorescence increase due to the hydrolysis of the peptide substrate (Mca–AVLQ ↓ SGFR-K(Dnp)K)[3]. The increasing additions of Zn^{2+} inhibit progressively the proteolytic activity of the enzyme. The fit of the kinetic data provided a K_i value of 0.58 ± 0.19 μM



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1. L. Zhang *et al. Science*, 2020, **368** , 409 —412
2. C. C. Lee *et al. FEBS Lett.*, 2007, **581** , 5454 —5458
3. Z. Jin *et al. Nature*, 2020, **582** , 289 —293

CONCLUSIONS

Zn^{2+} inhibits SARS-CoV-2 M^{pro} by binding at the active site that is ready to accommodate the metal as no significant structural rearrangement are observed. These results suggest that a Zn^{2+} coordinated to suitable ligands capable of interacting with additional sites on the protein surface could provide a significant increase in binding affinity, thus allowing the design of potent and more selective inhibitors of SARS-CoV-2 M^{pro}.

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