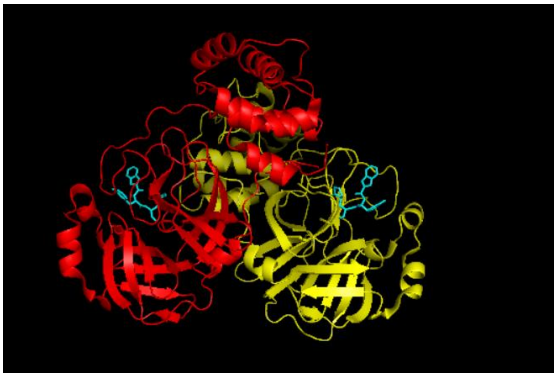
Practical Session 6: Mini-project

Mianyong Ding

R0823572

The novel coronavirus disease (SARS-CoV-2) caused the serious pandemic from 2019. SARS-CoV-2 main protease (M^{pro}) is cysteine protease which is essential for the replication of virus. The two main polyproteins (pp1a and pp1ab) translated from the RNA are cleavage by M^{pro} and papain-like proteases into 16 non-structural proteins. The non-structural proteins influence the translation of structural proteins by participating in the production of subgenomic RNA (Ramajayam *et al* ,2011).

The inhibition of the activity M^{pro} can be potential antiviral drug development. The inhibitor 11b to the structure of M^{pro} has been developed (Dai *et al* ,2020). And the COVID-19 main protease in complex with an inhibitor 11b (PDB ID: 6M0K) is loaded here to show the structure of M^{pro}.



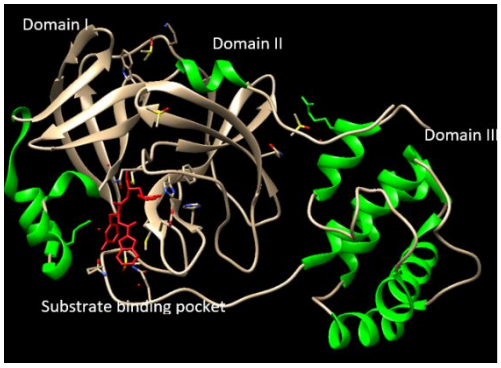


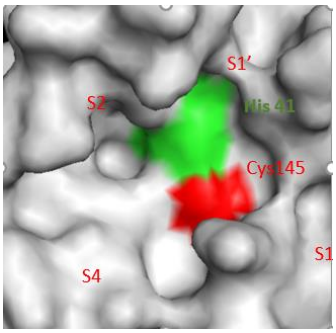
Figure 1(Left) The cartoon representation of M^{pro}.

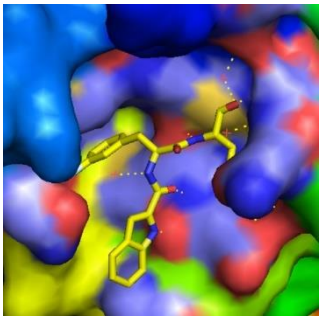
Figure 2(Right). The protomer of M^{pro}. The red sticks represent the 11b.

The active M^{pro} is a homodimer consisting of two protomers, and each protomer formed by three domains which are Domain I (residues 8–101), Domain II (residues 102–184), Domain III (residues 201–303) (Jin 2020).

The domain II has the antiparallel β-barrel structure with the compound of beta-sheet and beta-hairpin which can be observed from the figure 2. The covalent bond, hydrogen bond, beta-sheet, alpha helix,loops, beta -hairpin refer to the lecture 2 pdf page3,6, 8, 9 and 11. And the substrate-binding pocket site of SARS-CoV-2 M^{pro} is in the clef between domain I and domain II. And Cys145 and His41 (red and green regions) form a catalytic dyad as figure 3 shows. And the binding of substances for examplec11b can be seen from the figures 4. The formation of hydrogen bonds the C-S covalent bonds

between Cys145 and 11 blocks the substance binding and inhibitor the enzyme activity. The figure 3 also displayed the four regions of the binding pocket of M^{pro} (S1, S1',S2,S4). The electron density map shows 11b in the substrate binding pocket of SARS-CoV-2 M^{pro} forms a C-S covalent bonds with Cys145.





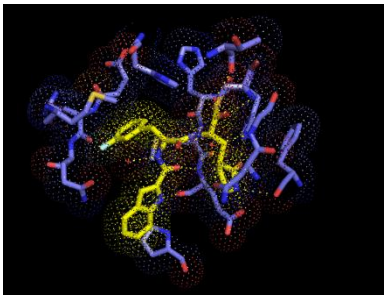


Figure 3(Left). The surface representation of M^{pro} binding pocket.

Figure 4(Middle). The representation of binding of 11b. The sticks represent 11b and the yellow dash is the hydrogen bond.

Figure 5(Right). The electron density map of the 11b and other interacted residues from M^{pro} .The yellow stick is 11b and the other purple sticks are residues from the M^{pro}.

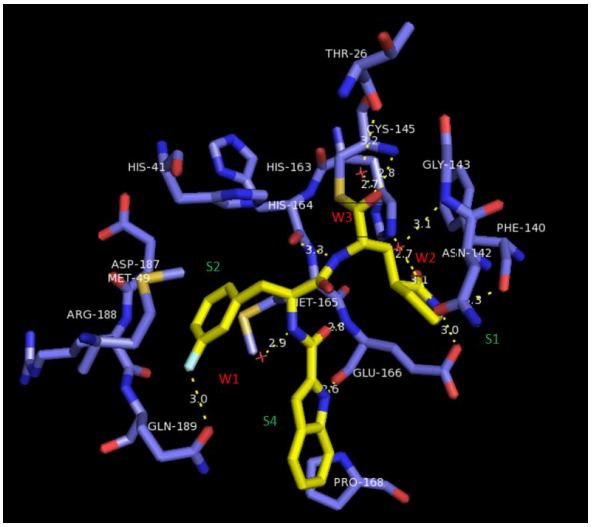


Figure 6. The mechanism of binding between 11b and M^{pro}. The yellow dashes represent hydrogen bonds and the red crossing stand for water molecular.

The 11b inhibitor inhibits the enzyme activity of M^{pro} through occupying the substrate-binding pocket. The mechanism of binding is displayed as figure 6, the yellow stick stands for the 11b. The region of protein for binding with the 11b is divided into four regions, S1', S1,S3 and S4. The water molecular w3 forms the hydrogen bonds with Thr26 (3.2 Å) and 11b (2.7 Å). The water molecular W2 forms hydrogen bonds with GLY143 (3.1 Å) and Asn 142(3.1 Å). And the amide bond of 11b is formed

hydrogen bond (2.9 Å) with the W1. The formation of hydrogen bonds between water and other residues can stable the 11b in the binding pocket.

The aldehyde group of 11 b forms a C-S covalent bond with the thiol of a cysteine residue, maintaining the inhibition activity of 11b. The Cys 145 also forms a 2.8 hydrogen bond with oxygen atom of 11b. The side chain of His163 forms a 2.7-Å hydrogen bond with (S)- γ -lactam ring of 11b. And the NH group of Phe140 and Glu 166 also forms 3.3-Å and 3.0-Å hydrogen bonds with (S)- γ -lactam ring of 11b. These three hydrogen bonds stabilize the (S)-γ-lactam ring. In the S3 site, the formation of 3.0-Å hydrogen bond between Gln 189 and 3-fluorophenyl group of 11b also stabilizes the 11b.

The Glu 166 interacts with amide bonds on the chain of 11b through 2.8 Å hydrogen bond. And the Glu 166 also forms a 2.6 Å hydrogen bond with the indole group of 11b. Pro168 and Gln189 interact with the indole group of 11b. The hydrophobic interactions between the aryl group and the side chains of residues His41, Met49, Asp187, Val186, Met165 and Arg188 also stabilize the 11b. The hydrophobic interaction refers to the lecture2 pdf page 7. As from the lecture, the hydrophobic interaction is the combination of many other interaction and it does not really exist, and it occurs between the polar groups and non-polar group. In this structure, the hydrophobic interaction can used to understand and develop the 11b. And the interaction between 11b and enzyme is competitive inhibition which refer to the lecture 6(theme 7) pdf page 11. The competitive inhibition of 11b is through the occupation of the binding site. So the original substances cannot be bound.

Because of potential of off-target side effects, the covalently bound inhibitors may have the limit of inhibition activity and the non-covalent binding between M^{pro} and inhibitor ML188 was developed and reported (Lockbaum *et al*,2021). SARS-CoV-2 Main Protease (M^{pro}) in Complex with ML188 (PDB ID: 7L0D) is loaded.

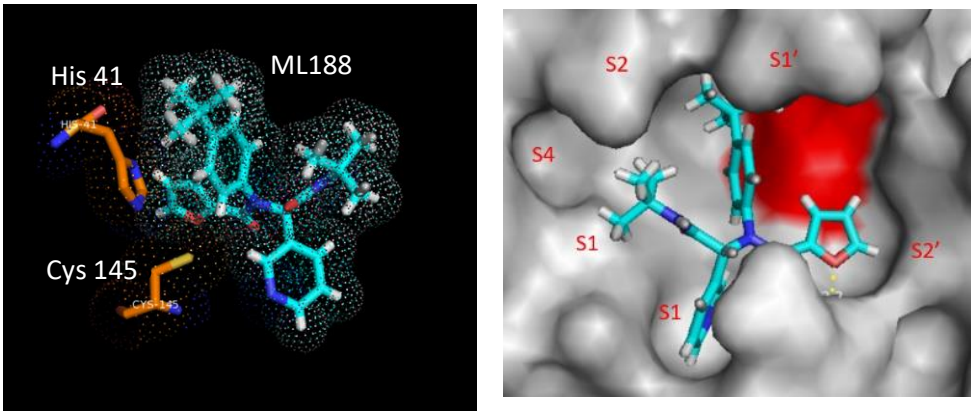


Figure 7(Left). Electron density map of ML188 and Cys 145 His 41.

Figure 8(Right). The ML188 and surface representation of M^{pro}.

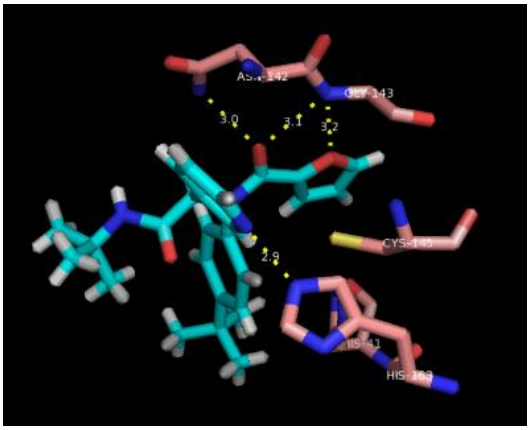


Figure 9. The interaction between ML188 and some residues from M^{pro}.

The figure 7 and figure8 show the non-covalent binding of ML188 at the binding pocket site of M^{pro}. The 3-pyridyl ring nitrogen forms a 2.9 Å hydrogen bond with the His-163. The backbone NH of Gly 143 interacts with the furan ring oxygen and the amide carbonyl oxygen through a bi-furcated interaction. Asn 142 forms a 3.0 Å hydrogen bond with the ML188.

The techniques are used here for example 11b structure is determined by 1H and 13C NMR(refer to the lecture 1,pdf page 5), High-resolution mass spectrometry (HRMS) and HPLC spectra. After crystallization, X-ray crystallography was used to measure the complex of M^{pro} and inhibitor. The X-ray crystallography and NMR was described in lecture 1 pdf page 4 ,5 and lecure 5 pdf page 2, which can helpful to understand the detection of structure. And the finally the structures were determined by molecular replacement (MR).

References:

Dai, W., Zhang, B., Jiang, X. M., Su, H., Li, J., Zhao, Y., Xie, X., Jin, Z., Peng, J., Liu, F., Li, C., Li, Y., Bai, F., Wang, H., Cheng, X., Cen, X., Hu, S., Yang, X., Wang, J., . . . Liu, H. (2020). Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. *Science*, 368(6497), 1331–1335. https://doi.org/10.1126/science.abb4489

Lockbaum, G. J., Reyes, A. C., Lee, J. M., Tilvawala, R., Nalivaika, E. A., Ali, A., Kurt Yilmaz, N., Thompson, P. R., & Schiffer, C. A. (2021). Crystal Structure of SARS-CoV-2 Main Protease in

Complex with the Non-Covalent Inhibitor ML188. *Viruses*, 13(2), 174. https://doi.org/10.3390/v13020174

Jin, Z., Du, X., Xu, Y. *et al.* Structure of M^{pro} from SARS-CoV-2 and discovery of its inhibitors. *Nature* **582,** 289–293 (2020). https://doi.org/10.1038/s41586-020-2223-y.

Ramajayam, R., Tan, K. P., & Liang, P. H. (2011). Recent development of 3C and 3CL protease inhibitors for anti-coronavirus and anti-picornavirus drug discovery. *Biochemical Society Transactions*, 39(5), 1371–1375. https://doi.org/10.1042/bst0391371.