

# Analyzing drug resistance in terms of substrate recognition in HCV NS3 protease

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## Background

Hepatitis C virus NS3 protease is essential to the viral lifecycle by cleaving at least four sites along the viral polyprotein, and for this reason, has been viewed as an attractive therapeutic target. Although several protease inhibitors have shown promise in clinical trials, drug resistance has been documented in both replicon studies and patient populations. From our previous studies of the balance of substrate recognition with the occurrence of drug resistance in HIV-1 protease, we found that most primary active site mutations do not extensively contact substrates, but are critical to inhibitor binding. We are extending this investigation to study drug resistance in HCV NS3 protease in the context of substrate recognition.

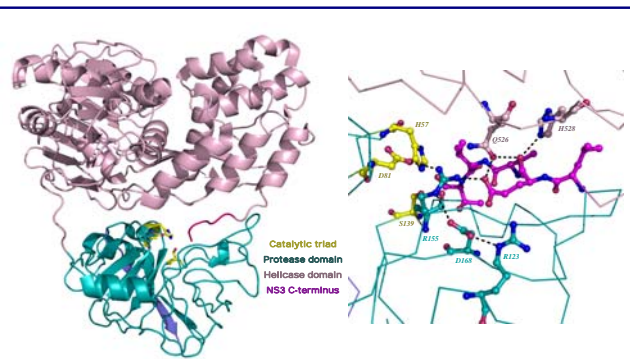


Figure 1: The 1CU1 structure of full-length NS3 (left) shows the C-terminus in the protease active site<sup>1</sup>. A close up view of this structure (right) reveals a hydrogen bonding network between residues of both the protease and helicase domains.

## Lessons from HIV-1 protease

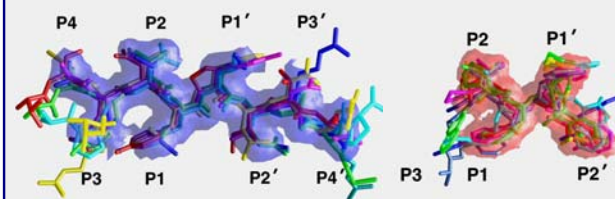


Figure 2: Superimposition of HIV-1 protease peptide substrates (left) and inhibitors (right) demonstrates a consensus volume defined as the "substrate-inhibitor envelope"<sup>2,3</sup>.

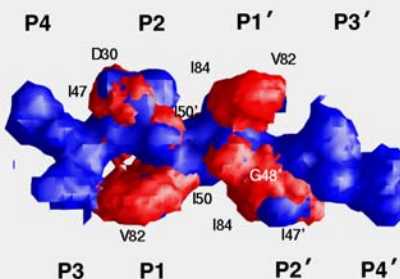


Figure 3: Superimposition of the HIV-1 protease substrate and inhibitor envelopes reveals areas where the consensus inhibitor volume deviates from the substrate envelope. These areas correspond to drug-resistance mutations commonly observed in treated patient populations<sup>4</sup>.

## Methods

Peptides corresponding to each of the three HCV NS3 substrates were modeled in the active site of full-length single-chain NS3 structure (1CU1). Crystal structures of NS3 protease in complex with inhibitors (2FM2, 20C8) were then superimposed to determine areas where the inhibitors extended beyond the van der Waals surface of bound substrates.

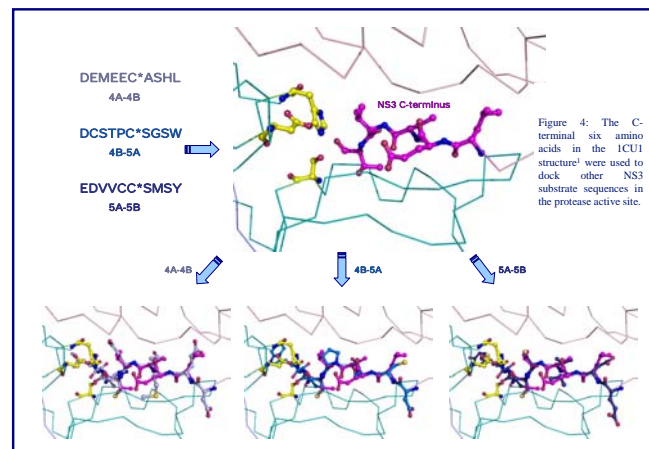


Figure 4: The C-terminal six amino acids in the 1CU1 structure<sup>1</sup> were used to dock other NS3 substrate sequences in the protease active site.

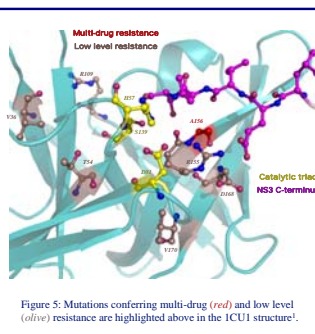


Figure 5: Mutations conferring multi-drug (red) and low level (olive) resistance are highlighted above in the 1CU1 structure<sup>1</sup>.

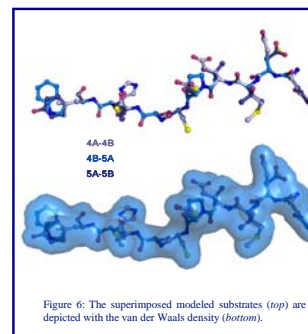


Figure 6: The superimposed modeled substrates (top) are depicted with the van der Waals density (bottom).

## Results

The molecular modeling of substrates in HCV NS3 provides a rationale that begins to explain a basis for drug resistance mutations to NS3 inhibitors at R155 and A156. In particular, substrate recognition is preserved when resistance occurs, as these residues appear to be making more extensive contacts with the inhibitors than they are making with the substrates.

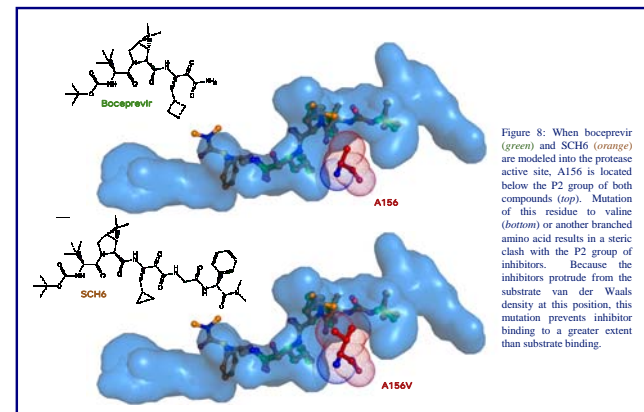


Figure 8: When boceprevir (green) and SCH6 (orange) are modeled into the protease active site, A156 is located below the P2 group of both compounds (top). Mutation of this residue to valine (bottom) or another branched amino acid results in a steric clash with the P2 group of inhibitors. Because the inhibitors protrude from the substrate van der Waals density at this position, this mutation prevents inhibitor binding to a greater extent than substrate binding.

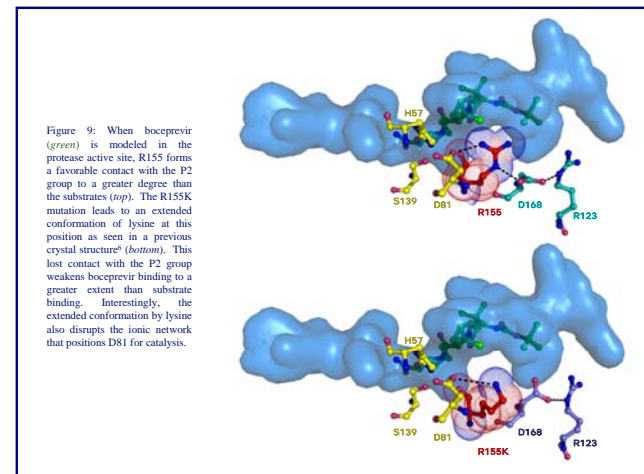


Figure 9: When boceprevir (green) is modeled in the protease active site, R155 forms a favorable contact with the P2 group to a greater degree than the substrates (top). The R155K mutation leads to an extended conformation of lysine at this position as seen in a previous crystal structure<sup>5</sup> (bottom). This lost contact with the P2 group weakens boceprevir binding to a greater extent than substrate binding. Interestingly, the extended conformation by lysine also disrupts the ionic network that positions D81 for catalysis.

## References

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## Conclusions

Similar to our findings for HIV-1 protease, resistance to current NS3 protease inhibitors appear to occur in a manner that maintains substrate recognition. This implies that future NS3 protease inhibitors that fit better within the substrate binding region should be less susceptible to drug resistant mutations.

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