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Evaluating Nirmatrelvir Resistance in SARS-CoV-2 Main Protease: A Comparison Between MM/PBSA and Free Energy Perturbation

Xiaoxiao Lyu[1] and Ye Mei[1, 2, 3]

[1]*State Key Laboratory of Precision Spectroscopy, School of Physics and Electronic Science, East China Normal University, Shanghai 200241, China*;

[2]*NYUE-CNU Center for Computational Chemistry at NYU Shanghai, Shanghai 200062, China*;

[3]*Collaborative Innovation Center of Eatreme Optics, Shanxi University, Taiyuan, Shanxi 030006, China.*

* Corresponding authors: ymei@phy.ecnu.edu.cn (Ye Mei)

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Abstract: Mutations that cause structural changes in proteins can sometimes reduce drug efficacy dramatically, a phenomenon known as mutation-induced drug resistance. For example, emerging drug-resistant mutations in the SARS-CoV-2 main protease (M^{pro}) threaten the long-term efficacy of nirmatrelvir, the active component of Paxlovid. Various methods have been developed to predict the impact of such mutations, with differing levels of reliability. In this study, comparative binding free energy calculations using Molecular Mechanics/Poisson–Boltzmann Surface Area (MM/PBSA) and Alchemical Transformation (also known as Free Energy Perturbation, or FEP) were performed to assess five naturally occurring M^{pro} mutations (SER144ALA, MET165ALA, GLU166ALA, HIE172ALA, and GLN192ALA) at the nirmatrelvir binding site. The results reveal a weak correlation ($R_{Pearson}$ = 0.18) between MM/PBSA predictions and experimental data. In contrast, FEP calculations using either the Multistate Bennett Acceptance Ratio (MBAR) or Thermodynamic Integration (TI) yield stronger linear correlations ($R_{Pearson}$ = 0.56 and 0.57, respectively). This study highlights the superior reliability of FEP in quantifying binding affinity losses due to drug resistance and underscores its potential for the proactive surveillance of clinical resistance mutations. Moreover, such insights are crucial for advancing antiviral drug development and guiding the design of inhibitors with a reduced risk of resistance evolution.

Key words: drug resistance, MM/PBSA, free energy perturbation, alchemical transformation, binding affinity.

1. Introduction

The emergence of antiviral resistance is prevalent in the treatment of chronic or persistent viral infections, including human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C (HCV), herpesviruses, and influenza [1]. In these cases, prolonged antiviral therapy, particularly as monotherapy, has driven the selection of viral escape mutations, consequently reducing therapeutic efficacy and leading to treatment failure [2-4]. Resistance to protease inhibitors arises from amino acid substitutions occurring either within the substrate-binding pocket

or at distal sites. At the molecular level, this resistance is primarily driven by a significant reduction in the binding affinity of the inhibitor for mutated protease, while substrate binding remains largely unaffected [5]. This change in affinity can be quantitatively assessed through binding free energy calculations. Various methods have been proposed for the calculations of binding free energies between ligands and their target proteins. Among these, Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) and Alchemical Transformations (also known as Free Energy Perturbation or FEP hereafter) methods are the most widely used in both academia and industry today [6-9]. However, each method has its own advantages and disadvantages in terms of the accuracy and computational cost.

Since December 2019, the COVID-19 pandemic caused by SARS-CoV-2 has severely impacted global health [10-12]. SARS-CoV-2 Main Protease (M^{pro}) plays a crucial role in viral replication by cleaving the viral polyproteins pp1a and pp1ab at 11 distinct sites, thereby generating nonstructural proteins essential for the viral lifecycle [4,13,14]. From an evolutionary perspective, the amino acid sequence and three-dimensional structure of M^{pro} are highly conserved across the subfamily *Coronavirinae*, providing a strong mechanistic foundation for the development of therapeutics, particularly in response to emerging SARS-CoV-2 variants with potential immune evasion concerns [10,15,16]. Moreover, the absence of a homologous protease in humans enhances the specificity and safety of targeting Mpro, making it an attractive and promising drug target for antiviral therapy development [17-21].

Pfizer's oral drug Paxlovid which combines the M^{pro} inhibitor nirmatrelvir and its metabolic enhancer ritonavir was granted Emergency Use Authorization by the FDA in December of 2021. Nirmatrelvir (PF-07321332) has been shown to be a highly effective inhibitor of M^{pro}, with an IC_{50} of 4 nM. Meanwhile, nirmatrelvir exhibits an extraordinary level of selectivity against a panel of human proteases, with submicromolar activity observed only for cathepsin K (IC_{50} = 231 nM), underscoring its potential as a therapeutic agent with minimal off-target effects [10,22]. The active site of SARS-CoV-2 M^{pro} features a non-canonical catalytic dyad, Cys145-His41, where the Sγ atom of Cys145 forms a reversible C-S covalent bond with the nitrile carbon of nirmatrelvir. In a study by Zhao *et al* [10]., the electron density map captured dual conformations of the catalytic cysteine, further supporting the reversibility of the covalent bond. Early administration of nirmatrelvir in COVID-19 reduces viral load and decreases the risk of progression to severe disease [23,24]. The ease of oral administration of nirmatrelvir further establishes it as a favorable option for high-risk patients [4].

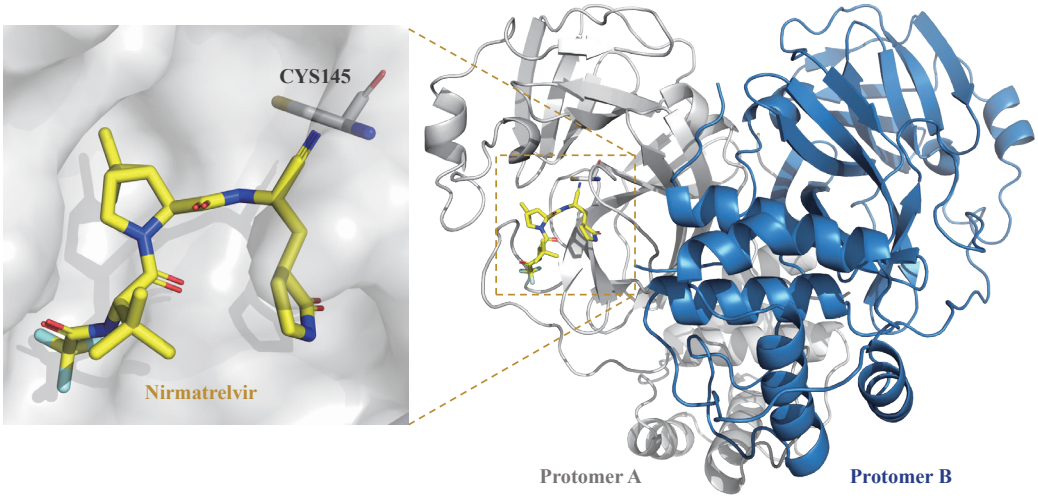

Figure 1: Nirmatrelvir (depicted as sticks) in complex with SARS-CoV-2 M^{pro}. The M^{pro} dimer is illustrated using a cartoon representation, with the two subunits colored gray and blue.

However, the emergence of drug resistance mutations in Mpro raises the concern of possible alter susceptibility of nirmatrelvir and threaten the long-term effectiveness as an antiviral treatment for potential future pandemic. Several studies have reported the emergence of nirmatrelvir-resistant M^{pro} mutants through viral passage experiments. Mutations in residues SER144, GLU166, and ALA173 have direct impact on nirmatrelvir inhibition [25-29].

In this study, MM/PBSA and FEP were applied to calculate the binding free energies of nirmatrelvir with Mpro mutants (SER144ALA, MET165ALA, GLU166ALA, HIE172ALA, and GLN192ALA), as well as the wild-type M^{pro}. These mutants were selected based on prior experimental evidence indicating their role in conferring resistance to nirmatrelvir [29]. Accordingly, the correlation between the calculated binding free energies and experimental affinity values was evaluated using the Pearson correlation coefficient. Notably, only the binding energy differences along the sequence were examined, and entropy contributions were assumed to be invariant across the protein variants and thus were omitted in the MM/PBSA calculations. The Pearson correlation coefficient of 0.18 from the MM/PBSA calculations shows a weak linear correlation with the experimental data. In contrast, FEP calculations using MBAR and TI for the alchemical transformation analysis yield correlation coefficients of 0.56 and 0.57, respectively, indicating a better agreement with the experimental data. The study suggests that the FEP calculations strongly correlate with experimental results, highlighting the potential of the FEP method as a powerful tool for providing accurate affinity values between protein and ligand, particularly when considering binding energy differences along the sequence. Meanwhile, the MM/PBSA calculations remain valuable (probably for pre-screening) due to their lower computational cost, especially when precise agreement with experimental absolute affinities is not critical.