Non-Active Site Mutants of HIV-1 Protease Influence Resistance and Sensitization Towards Protease Inhibitors

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Alchemical binding free energy change calculation; Distant site mutations; HIV-1

protease inhibitors; Hydrogen bond network perturbation; Resistance-associated mutations

Abstract

Background

HIV-1 can develop resistance to antiretroviral drugs, mainly through mutations within the target regions of the drugs. In HIV-1 protease, a majority of resistance-associated mutations that develop in response to the therapy with protease inhibitors are found in the protease's active site that serves also as a binding pocket for the protease inhibitors, thus directly impacting the protease-inhibitor interactions. Some resistance-associated mutations, however, are found in more distant regions, and the exact mechanisms how these mutations affect protease-inhibitor interactions are unclear. Furthermore, some of these mutations, e.g. N88S and L76V, do not only induce resistance to the currently administered drugs, but contrarily induce sensitivity towards other drugs. In this study, mutations N88S and L76V, along with two other resistance-associated mutations, M46I and I84V, are analysed by means of molecular dynamics simulations to investigate their role in complexes of the protease with different inhibitors and in different background sequence contexts.

Results

Using these simulations for alchemical calculations to estimate the effects of mutations M46I, I84V, N88S, and L76V on binding free energies shows they are in general in line with the mutations' effect on IC50 values. For the primary mutation L76V, however, the presence of a background mutation M46I in our analysis influences whether the unfavourable effect of L76V on inhibitor binding is sufficient to outweigh the accompanying reduction in catalytic activity of the protease. Finally, we show that L76V and N88S changes the hydrogen bond stability of these residues with residues D30/K45 and D30/T31/T74, respectively.

Conclusions

We demonstrate that estimating the effect of both binding pocket and distant mutations on inhibitor binding free energy using alchemical calculations can reproduce their effect on the experimentally measured IC50 values. We show that distant site mutations L76V and N88S affect the hydrogen bond network in the protease's active site, which offers an explanation for the indirect effect of these mutations on inhibitor binding. This work thus provides valuable insights on interplay between

primary and background mutations and mechanisms how they affect inhibitor binding.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed.

However, the manuscript can be downloaded and accessed as a PDF.

Figures

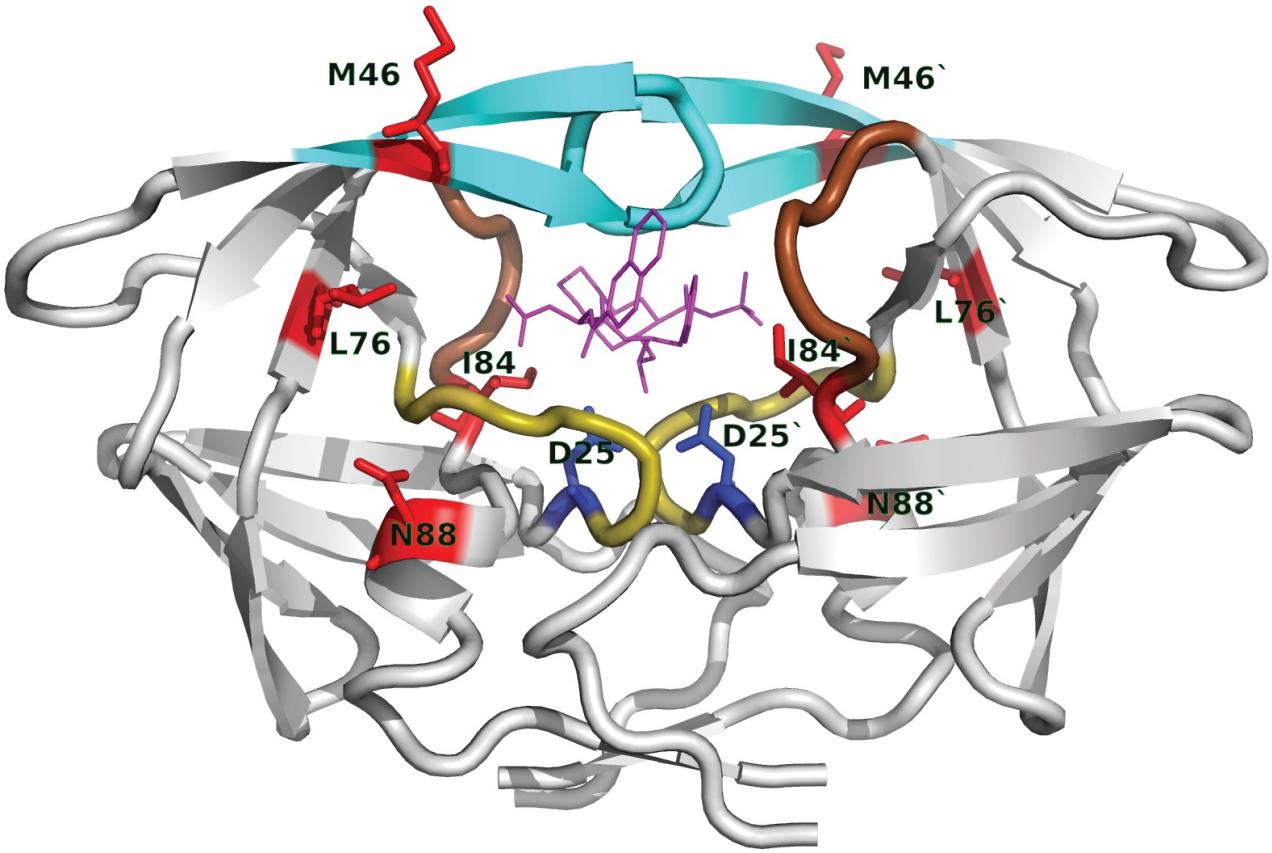

Figure 1

HIV protease structure. Flap region in cyan, 80s loop in brown, active-site proximate loop in olive colours. Mutations analysed in this study (red), catalytic site residue (blue) and bound inhibitor (magenta) are shown in sticks model.

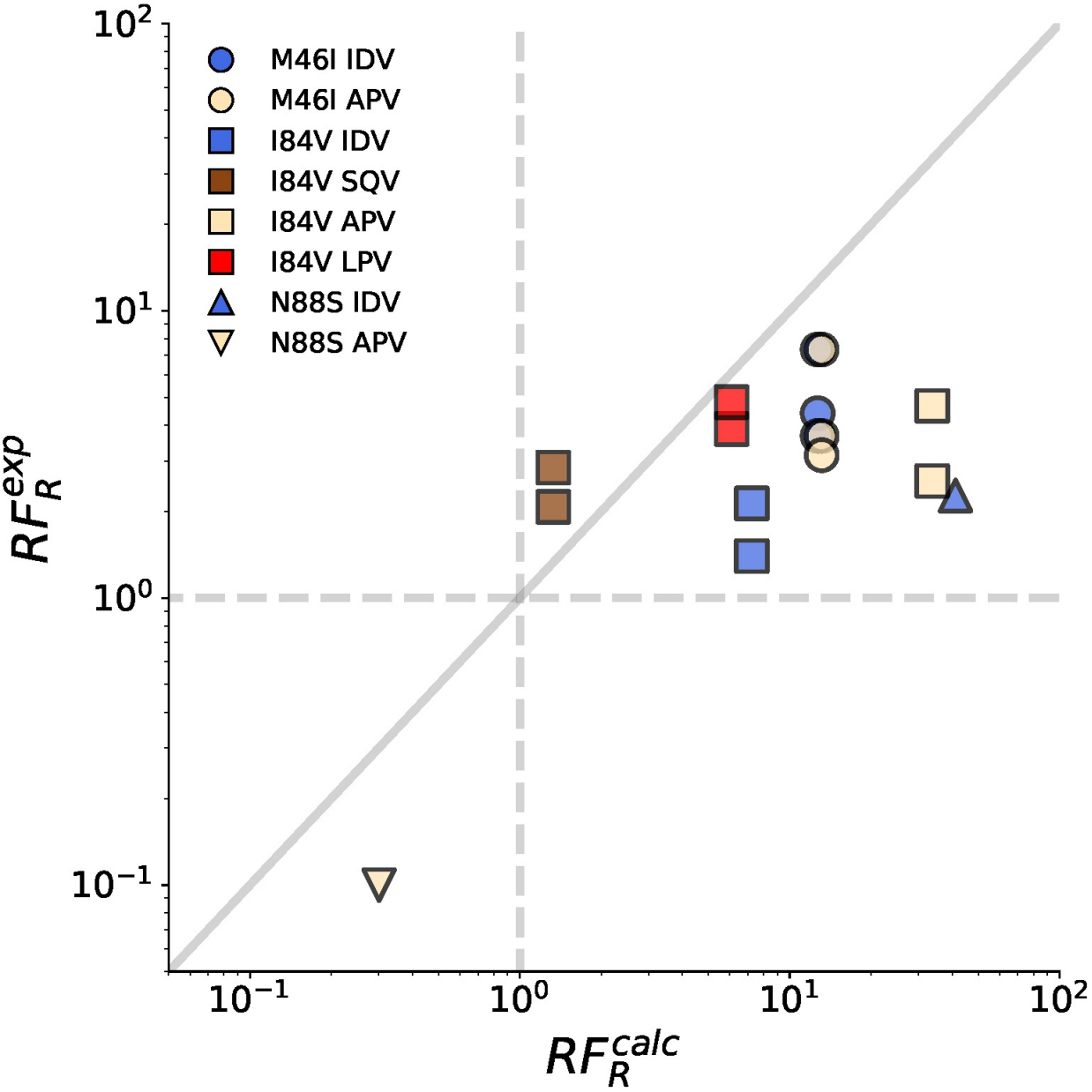

Figure 2

Predicted and experimental RF measurements. Each symbol corresponds to a unique sequence background and colour corresponds to inhibitor. Nota bene: in case of APV, RF exp measurements are for its prodrug FPV

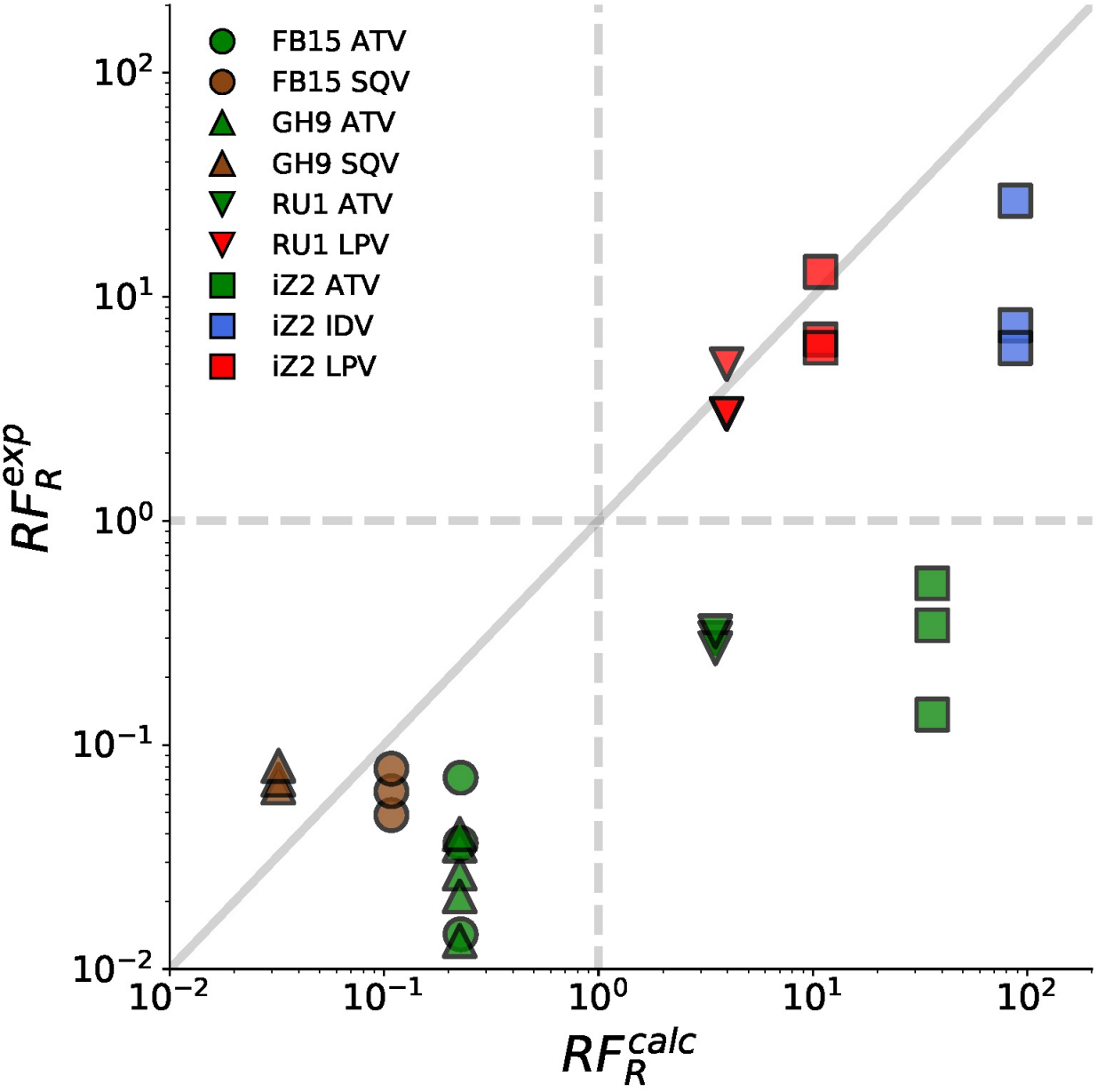

Figure 3

Predicted and experimental RF measurements. Each symbol corresponds to a unique sequence background and colours correspond to inhibitor.

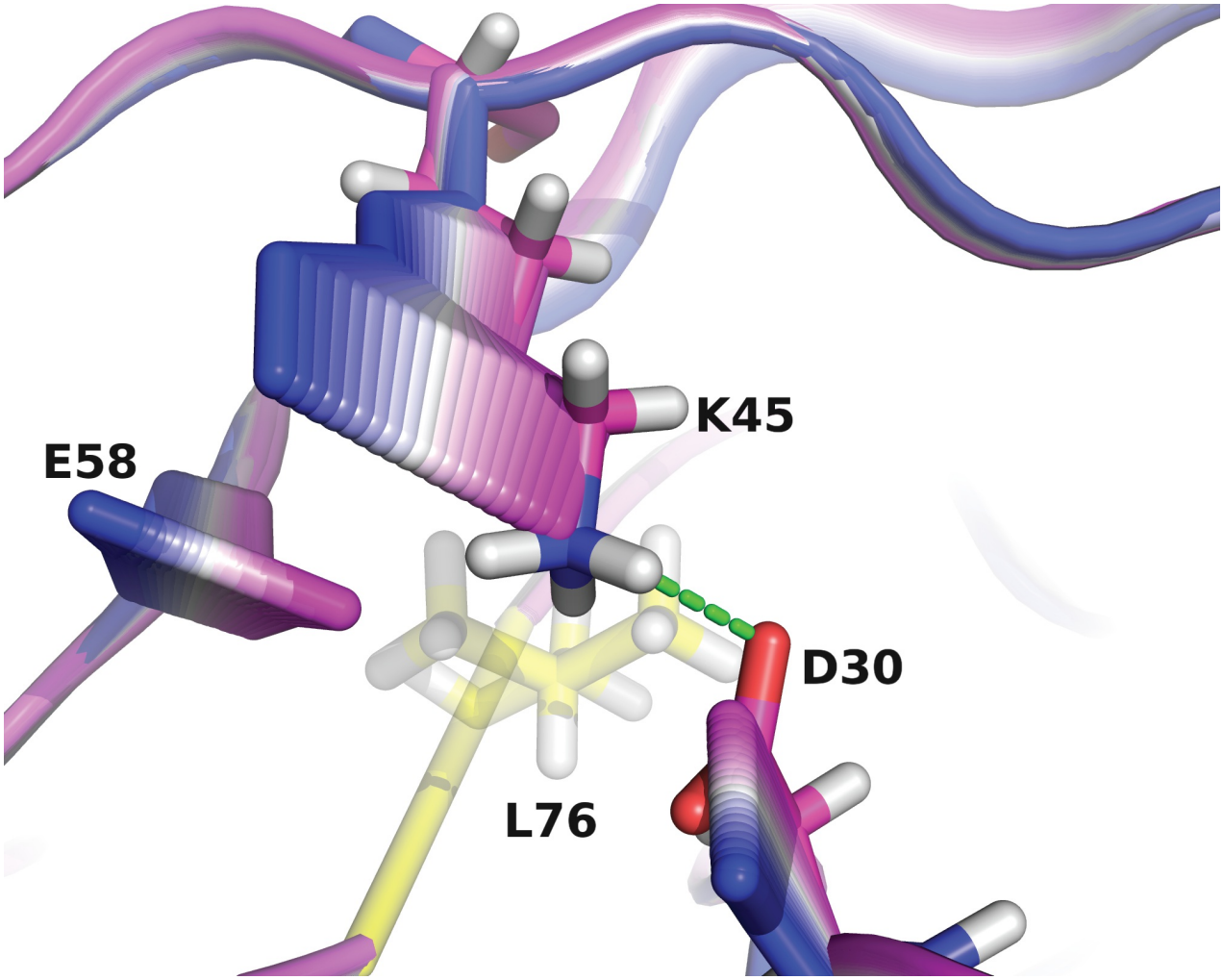

Figure 4

Interpolation between the extremes of the FMA models for the protease (genotype RU1) in complex with LPV. Blue-to-magenta bands correspond to the interpolation along the mode as represented as cartoon for backbone and as sticks for residues 30, 45, and 58, with blue corresponding to L76 state and magenta to V76 state. Green dashed line represents a hydrogen bond between residues D30 and K45. Mutated residue 76, here semi-transparent in yellow, as well as hydrogen atoms, here in gray, were not part of the FMA models and are here for representational purposes only.

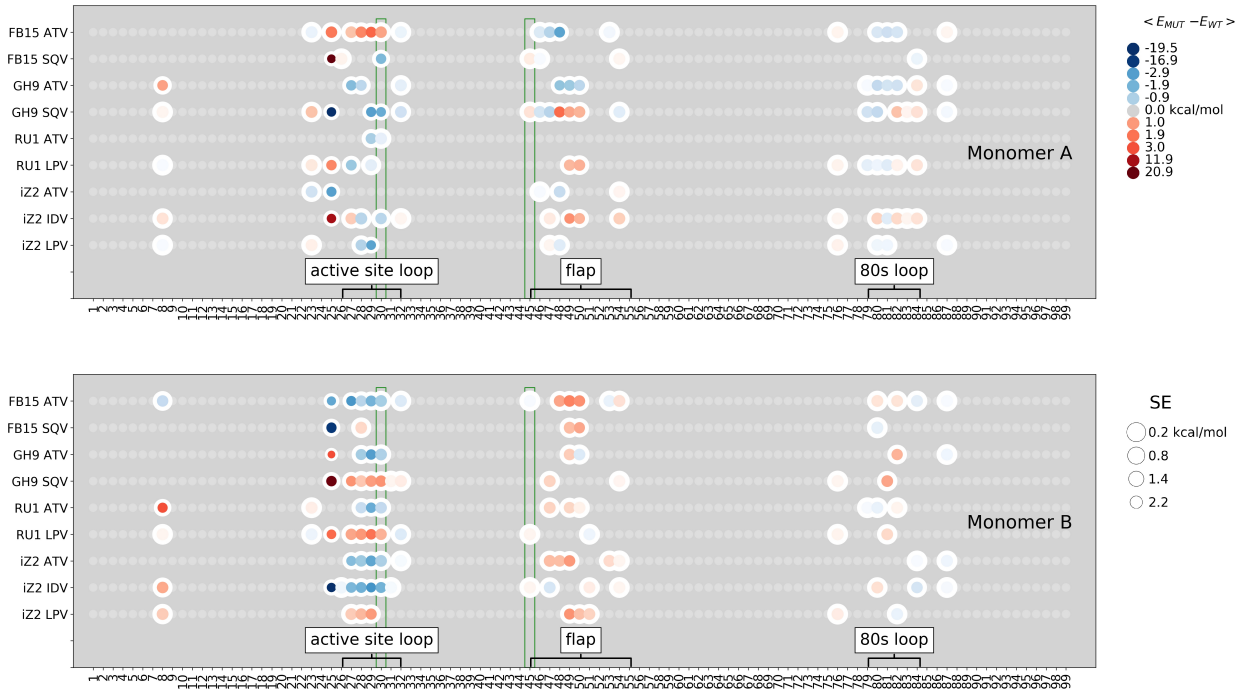

Figure 5

Energy differences of non-bonded interaction between protein and in-hibitor in wildtype and mutant complexes. Residues, for which the difference (EMUT − EWT) between the wildtype and the mutant complexes is higher than the propagated error (SE) and its absolute value higher than 0.1 kcal/mol, are rep-resented as a colored circle, where the color represents relative interaction energy and the size of the circle relates inversely to the standard error of the estimate. Residues' 30 and 45 interactions are highlighted in green box.

Supplementary Files

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