Effect of D30N on Nelfinavir binding in HIV-1 protease as examined by hydropathic interactions (HINT)

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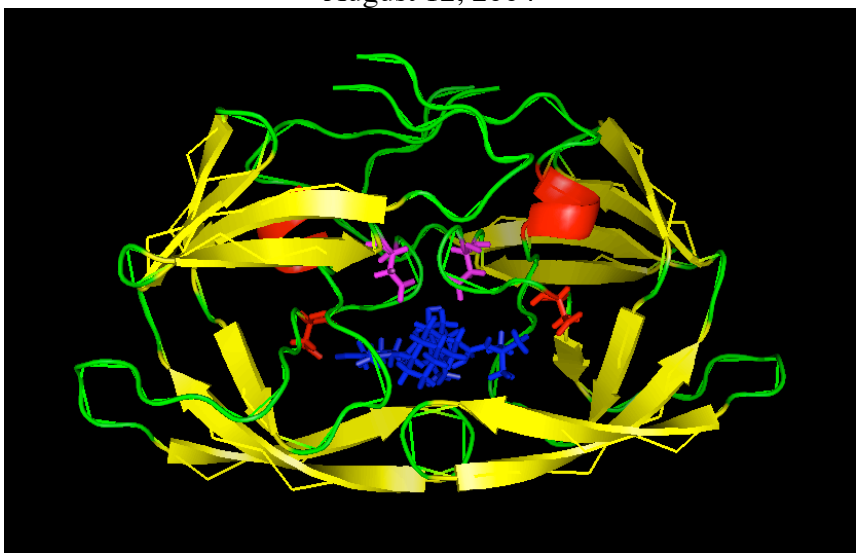


Abstract

The D30N mutation results in Nelfinavir resistance in the HIV-1 protease. The use of hydropathic interaction (HINT) calculations as a quantitative measure of free energy can show specific atom-atom interactions that become more or less favorable in the presence of a mutation. These calculations show that the mutated residue results in the loss of 230 and 38 hint units from interactions involving the one atom that is altered by the mutation.

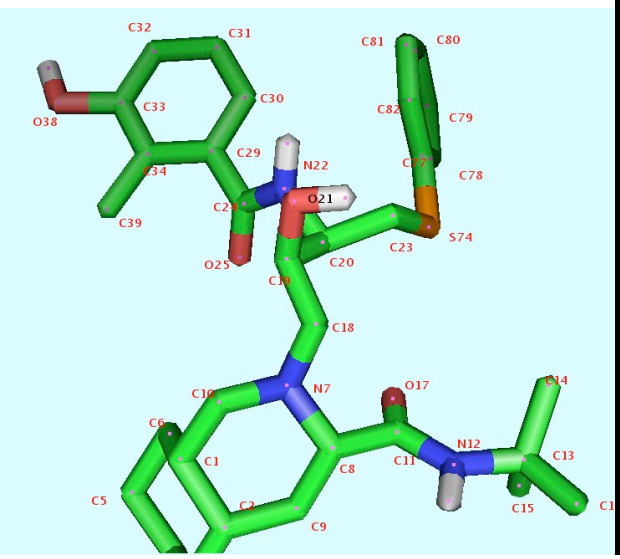
Introduction

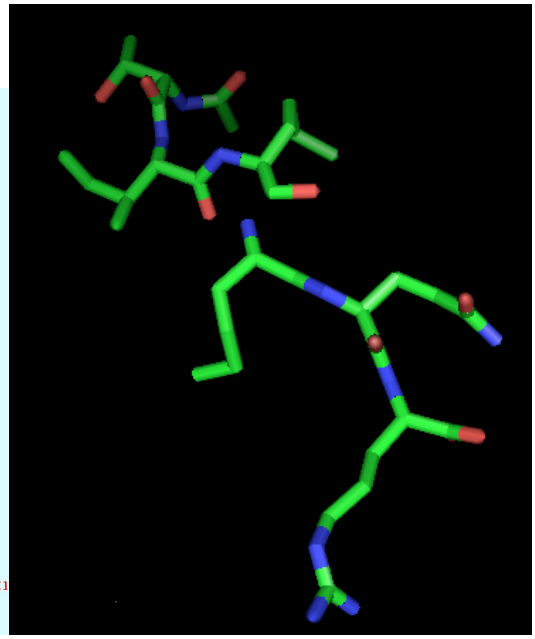
The human immunodeficient virus (HIV) requires the activity of a 198-residue homodimeric (two identical subunits, each with 99 residues) aspartic protease for the successful replication of infectious virons. The HIV-1 protease (PR) processed the *gag* and *gag-pol* polyproteins into usable subunits. There are currently several protease inhibitors (PIs) that are used to treat HIV/AIDS. The PIs work through competitive inhibition. However, due to the quick and highly error-prone replication of HIV, strains can select for polymorphisms that confer drug resistance in a very short period of time.

One PI used to treat HIV is Nelfinavir (also know as Viracept and AG1343). Nelfinavir resistance is conferred by a mutation at residue 30; Aspartic acid (ASP, D) is mutated to Asparagine (ASN, N). This changes a carboxylic acid to an amino group—an oxygen atom is changed into a nitrogen atom. Nelfinavir resistant mutants show little to no cross-resistance to other PIs. Nelfinavir resistant mutants retain catalytic activity while reducing affinity for Nelfinavir to the point where it is no longer effective.

The hydropathic interactions (HINT[1]) model provides empirical calculations of the non-covalent interaction force field. These calculations are heavily based upon experimental data for the portioning coefficient between octanol-1 and water ($LogP_{o/w}$). This includes (but is not limited to) hydrogen bonding, acid-base, hydrophobic-hydrophobic, acid-acid, base-base, and hydrophobic-polar interactions. A HINT "score" is calculated for the interaction between each pair of atoms. HINT is available as an addition to the molecular modeling program Sybyl (Tripos, Inc). This score can be translated into an estimate of the free energy. A positive score reflects a favorable interaction (negative free energy).

By analyzing the effect of the D30N mutation *in silico*, we will gain insight into what causes drug resistance. This understanding may lead to the development of new drugs that will continue to be effective in the presence of this mutation.





Picture 1: Nelfinavir *Picture 2: p2-NC*

Both pictures are shown in approximately the same spatial configuration so that accurate conclusions can be drawn between structures containing the other molecule.

Materials and Methods

Preparing the structures

Structures were obtained from the RCSB Protein Data Bank (http://www.rcsb.org). The following table summarizes the structures used in the experiment.

Table 1.

Structure	Mutations	Substrate
1OHR	none	Nelfinavir
1FGC	Q7K, L33I, L63I, C67A, C95A	p2-NC
1FFI	D30N, Q7K, L33I, L63I, C67A, C95A	p2-NC

p2-NC is a substrate analog (Acetyl Thr-Ile-Nle-r-Nle-Gln-Arg). All three structures contain different quantity of water molecules.

A structure was read into Sybyl. All three structures were examined in their unmutated states. Additionally, D30N or N30D mutations were performed. Additional

structures of 1FFI and 1FGC were attempted (to undo the five crystallization mutations, however time did not permit accurate results; one such "true wild type" structure was completed). Mutations were done, when needed, through the mutate monomer function (biopolymer>modify>). Hydrogen atoms were added to the structure using the biopolyer>add hydrogens command (all hydrogen, random orientation). Any mutations were optimized via the minimize subset command. Then the hydrogen atoms were optimized with the minimize command. The substrate/drug was extract from the molecule and placed into a second molecule file (and then deleted from the original file). The water molecules (selected from the substructure menu) were extracted (into a third file) and deleted in the same manner. All three molecules are named using simple notion (hint does not like long name or non-alpha characters).

HINT Calculations

The directions in Sybyl's TriposBookshelf were followed to do the HINT calculations. All HINT functions were done under the options menu. Partition maps were calculated for each molecule. Then an Intermolecular HINT Table was performed with the protease as molecule 1, the ligand and molecule 2, and the waters as the cofactor (molecule 3). The cut-off radius was changed to 6.00 A.

Results

From previous experiment[2], it is known that the wild type protease as well as the D30N mutant will interact with the natural substrate. However, Nelfinavir interacts less favorably with D30N than the wild type. Accordingly, we would expect to see HINT scores will little change in structures with the substrate analog and lower scores when Nelfinavir is with theD30N mutation. The following structures were examined and will be hence forth noted by number (in the left column).

Table2: Structures Examined with HINT

Assigned #	Starting Structure	Mutations	Sybyl Mutations
1	1FGC	Q7K, L33I, L63I, C67A, C95A	none
2	1FGC	Q7K, L33I, L63I, C67A, C95A	D30N
3	1FFI	D30N, Q7K, L33I, L63I, C67A, C95A	none
4	1FFI	D30N, Q7K, L33I, L63I, C67A, C95A	N30D
5	1FFI	Q7K, L33I, L63I, C67A, C95A	K7Q, I33L, I63L, A67C, A95C
9	1OHR	none	none
10	1OHR	none	D30N

Analysis

All interactions involving residues 30 and 130 were copied into another file. Then, interactions with a HINT score greater than or equal to 75 were recorded. This allowed for easier comparisons to be made and helped to distinguish "noise" in the calculations from true changes in values. The data was then graphed using Microsoft Excel (figures 1 through 4).

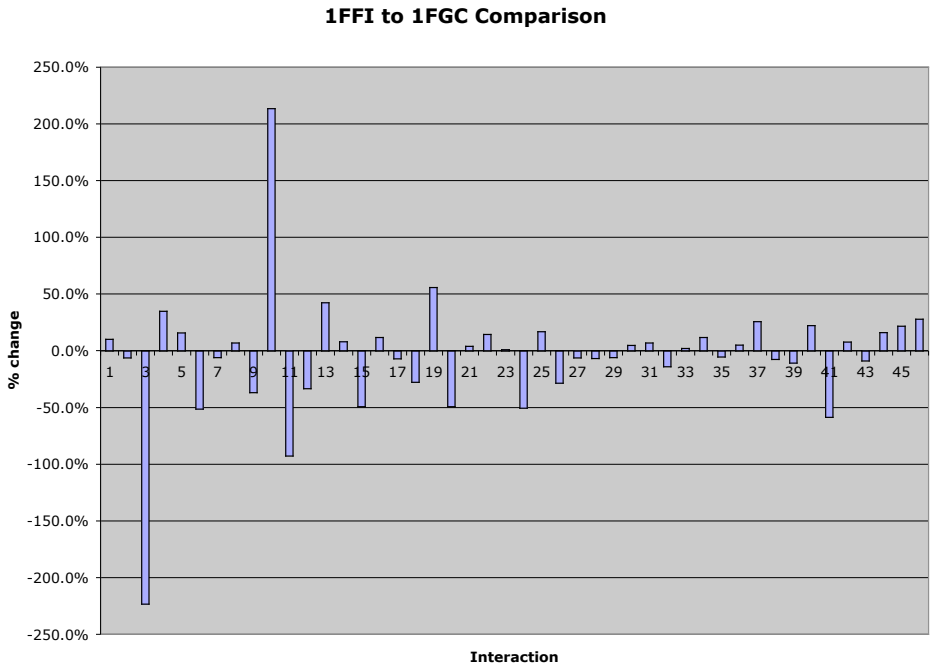


Figure 1: #1 versus #3 (above)

This compares the wild type with the D30N mutant directly from the crystal structures. The first thirteen interactions are from residues 30 and 130 (and thus may be "noise" since they do not all contain HINT scores greater than 75 in absolute value).

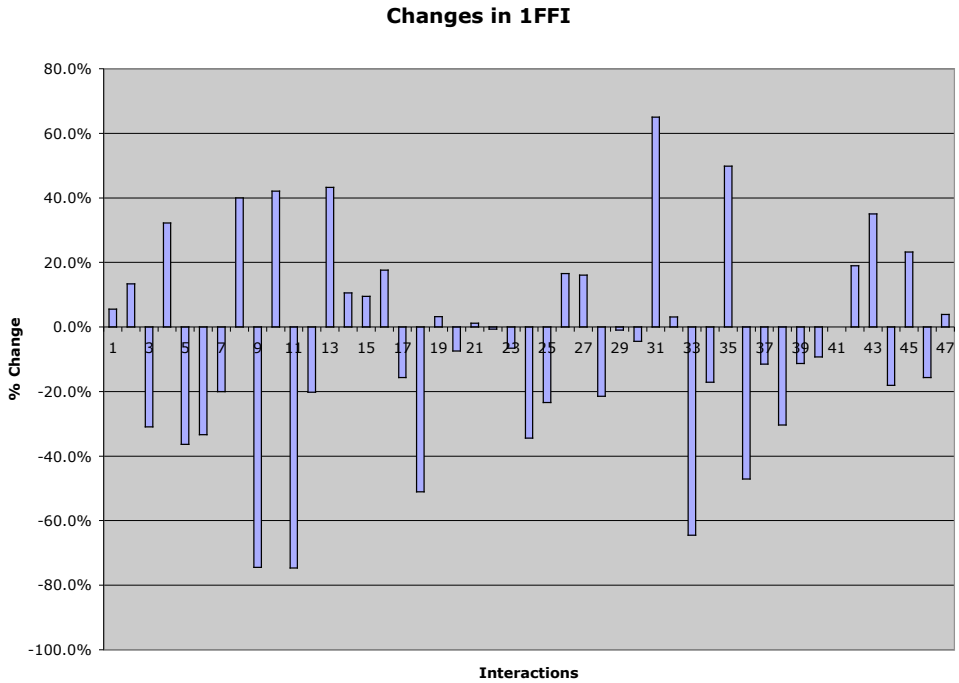


Figure 2: #1 versus #2 (above)

This figure compares the wild type and mutant forms starting with the 1FGC crystal structure.

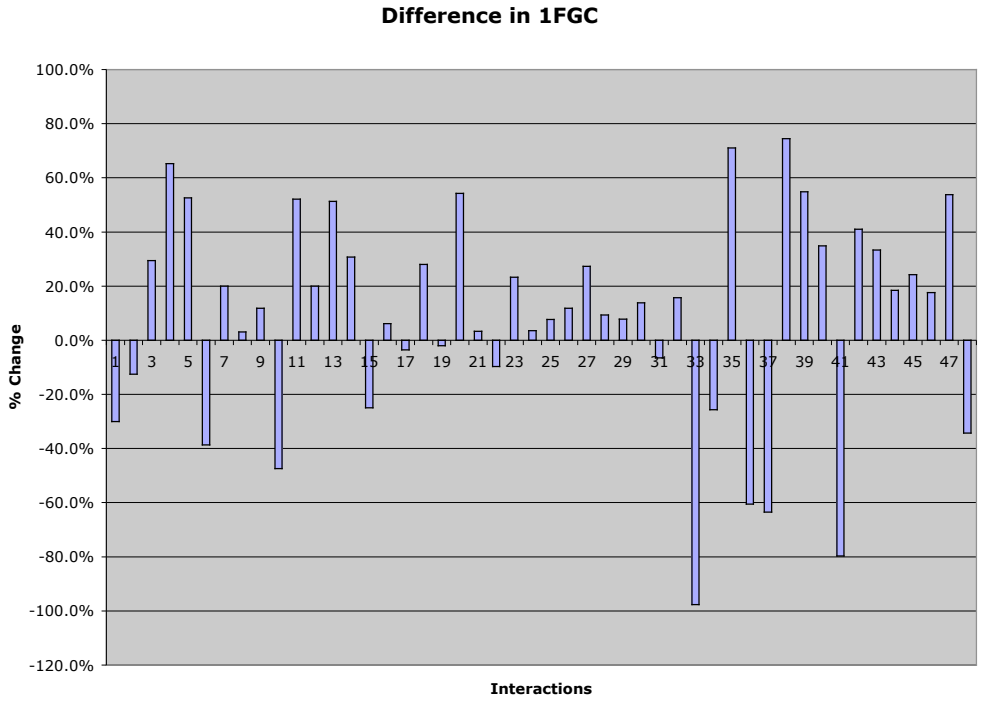


Figure 3: #3 versus #4 (above)

This figure compares the wild type and mutant forms starting with the 1FFI crystal structure.

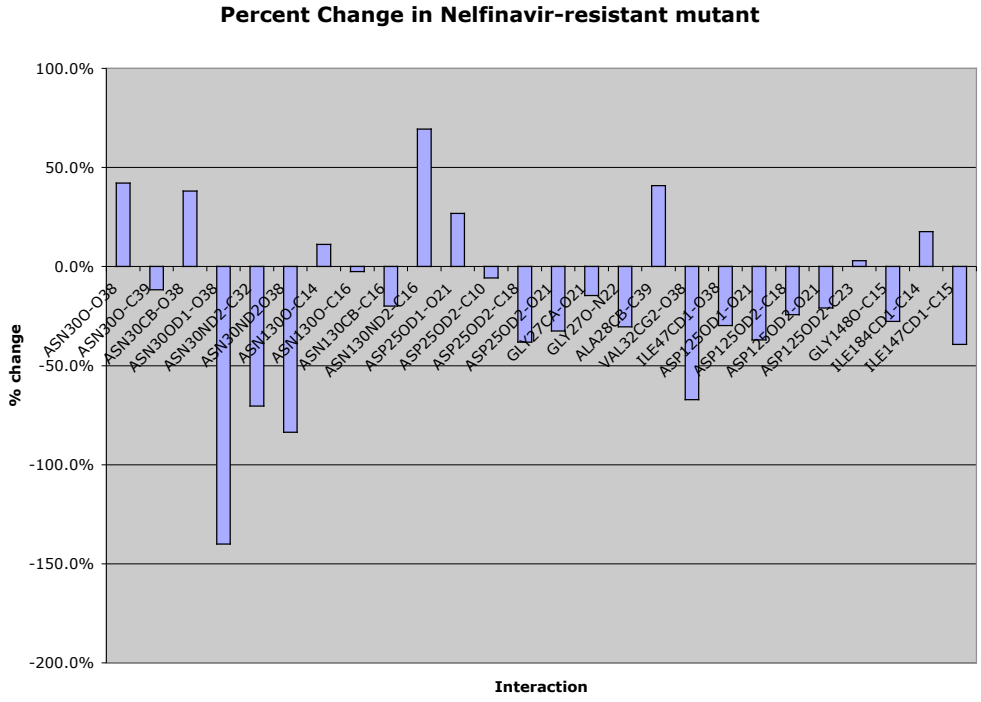


Figure 4: #9 versus 10 (above)

This figure compares the wild type and mutant forms of the protease bound to Nelfinavir, starting with the 1OHR crystal structure. The interactions are labeled.

Residues 30 and 130 are labeled as the mutant form. ASP30/130 OD2 is assumed to be analogous to ASP30/130 ND2.

Discussion

The D30N mutation did not have an overwhelming effect on any of the structures compared in this experiment. Figure 1 shows that changes are fairly random and that no single change (or small group of changes) confers a drop in favorable intereactions. Figures 2 and 3 show that large swings in scores are often "cancelled out" by opposing changes on nearby atoms on the same residue as well as the random up and down changes.

Data from structure #5 was nearly identical to that of #3 (data not shown). There were only a few differences (one or two hint units each). This is expected because the only difference between the structures is the five crystallization mutations. These mutations are said to not confer any structural difference[2].

On the other hand, the structures containing Nelfinavir show a trend toward lower hint scores. This means that the free energy is increasing and the interaction between PR and Nelfinavir is becoming less favorable. The largest such change is between the mutated atom in residue 30 and O38. The oxygen of ASP30 yields a hint score of 275 but the nitrogen of ASN30 only scores a 45. This is an 83.6% drop (230 hint units). There is also a loss of 38 hint units between OD2/NH2 and C32. This means that 268 hint units are lost when residue 30 is mutated from aspartic acid to asparagines. The beta carbon of residue 30 shows a loss of 42 hint units (interacting with O38 as well). The catalytic ASP125 also shows a large decrease in hint score, going from 491 to 388. This 103 unit drop is a 21% reduction in the interaction's score. Similarly, ASP25 has a 64 unit drop between OD2 and O21 (32.5%). The overall interaction constant drops from 9.89×10^2 to 3.38×10^2 as well. This is nearly a three-fold decrease in interaction score.

Acknowledgements

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Additional Information: Complete HINT calculation results are available upon request as well as fully detailed optimization techniques and parameters.

References

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