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BIN515 Final Take Home

1. a) The hydrophobic residues (ALA, PRO, VAL, LEU) have been chosen accordingly. The rest is considered to have hydrophilic properties. In order to look at their distribution, their ASA is calculated. Figure 1 shows the distribution of chosen amino acids.

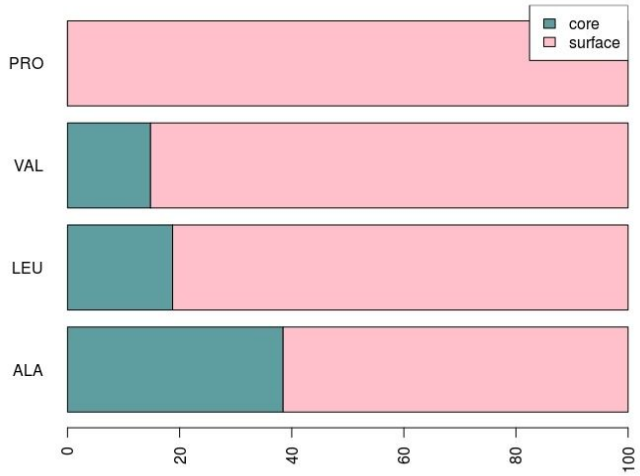
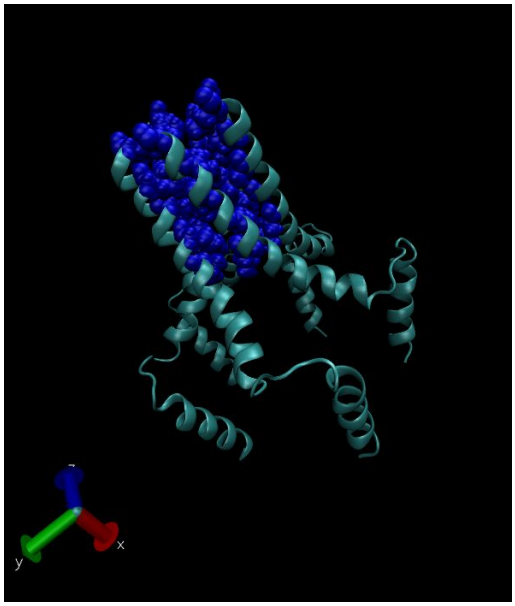


Fig1. Distributions of hydrophobic residues of 1h68

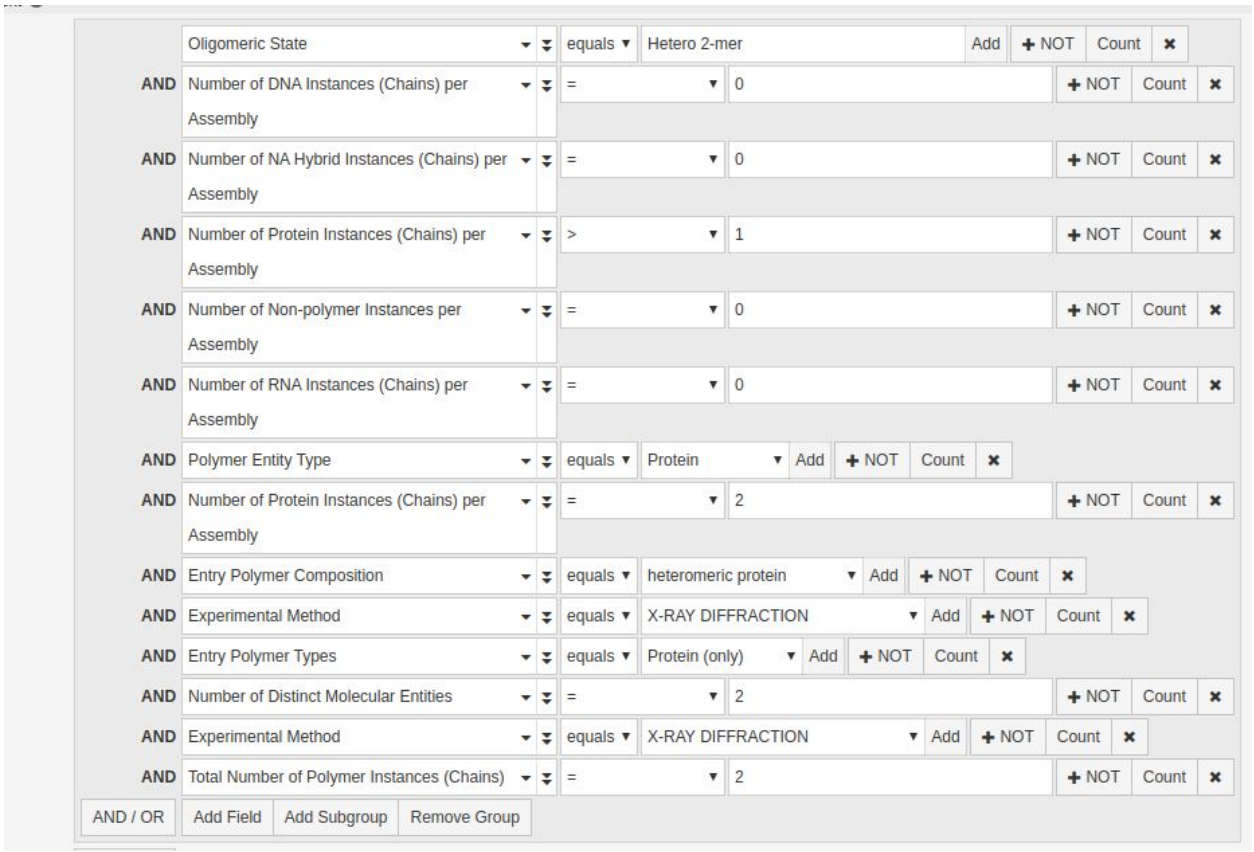
As seen on the barplot majority of the hydrophobic residues are located on the surface. However, generally proteins are suspended in water, therefore one may claim that hydrophobic residues should not be seen on the surface. In the case of 1h68, since it is a transmembrane protein, the surface of the protein is mainly exposed to the hydrophobic lipid environment in the bilayer as opposed to aqueous environment in the cytosol or extracellular matrix. Hence, justifying the overrepresentation of hydrophobic residues on the surface.

b) In the study conducted by Simmermann et al it is found that,

Leucine and alanine residues at the stem of monomers (shown in blue in below figure) are playing a key role in stabilizing the pentamer structure of the protein. This is shown by disruption of the pentamer after mutating those residues into ALA or PHE residues. Since ALA is a strong helix former, the authors claim that the disruption of the pentamer does not stem from the disruption of the secondary structure of the protein. Instead, they hypothesize that the stable pentamer structure is because of previously well described leucine/isoleucine heptad patterns (leucine/isoleucine residues that are each 7 residues apart). With this structure, monomers are anchored to each other thanks to interactions between aliphatic side chains of leucine/isoleucine residues and the fact that these appear in alternated fashion between monomers causing in zipper-like close physical interactions. Furthermore the authors underline that mutating the neighboring residues of stabilizing amino acids have no effect on quaternary structure, which again supports the claim that loss of stabilization does not stem from breaking of secondary structure, but rather disruption of leucine/isoleucine heptad patterns. [1]



2)



In order to find 20 heterodimers in PDB, the search criteria above is used. First 20 PDB ID is retrieved from the advanced search. PDB structures using command line downloader wget. (GNU/Linux) Subsequent to the downloading the 20 structures, files have been batch processed using a custom shell script which does the following:

- Two proteins separated into two distinct files

- Both separate structures together with the complex structure submitted to getArea server using the provided perl script

a) This step is implemented in the findinterface.py which does the following for each structure:
   i) Parse the getArea result
   ii) Calculate the difference between residue level ASA values of the proteins for complex and separate states to determine the interface residues (using cutoff 1 A^2)

b)

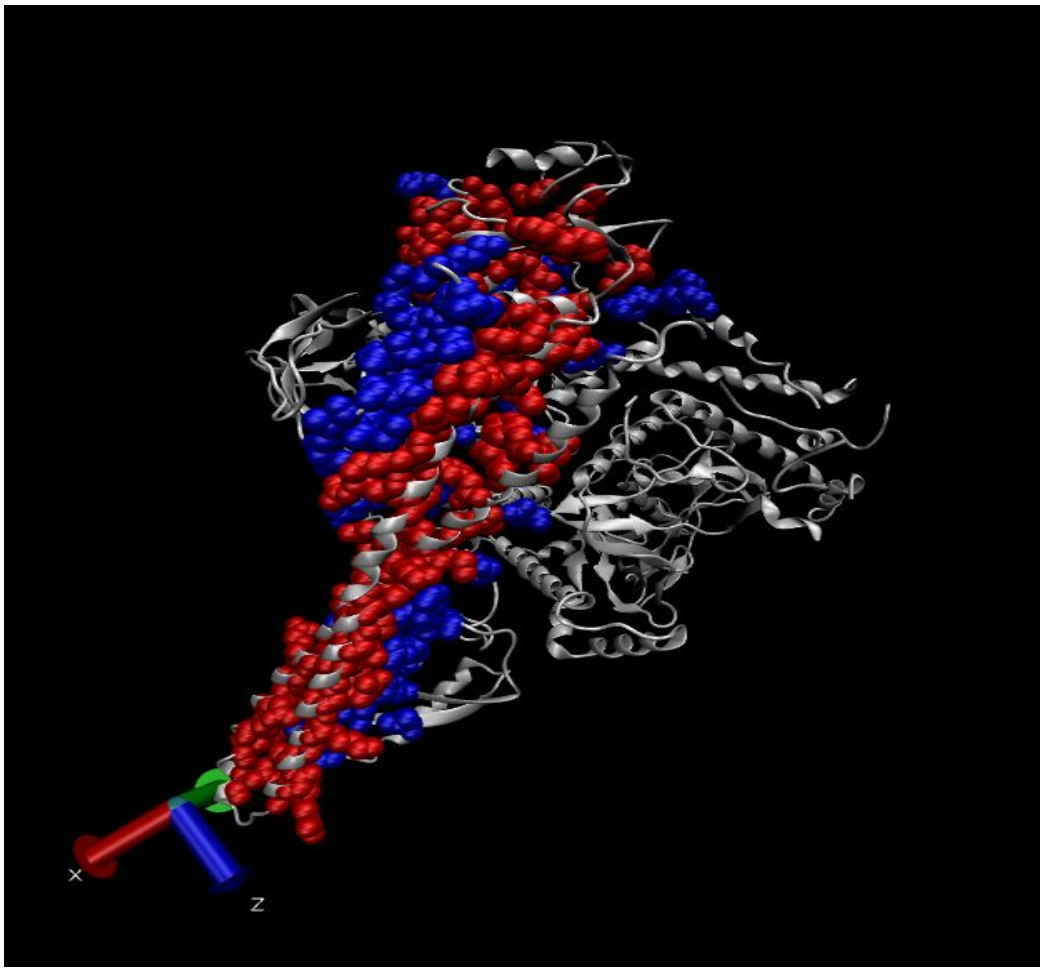


Fig. The interface region of 3HIZ is shown in vDW, the rest of the structure is shown in New Cartoon

c)

The highest propensity groups are positively charged amino acids and amino acids with aromatic side chains. This is due to higher capacity of those type of residues for forming hydrogen bonds and salt bridges.

Amino acid	Propensity
SER	1.07
TYR	2.076
HIS	0.581
ASP	0.996
ILE	0.432
PRO	1.309
THR	0.744
GLU	1.078
GLY	0.611
LEU	0.915
TRP	2.309
MET	0.941
ARG	1.498
VAL	0.842
CYS	0.285
ASN	1.31
ALA	0.925
LYS	1.196
PHE	1.589

GLN	0.678

Table 1. Propensity values of amino acids

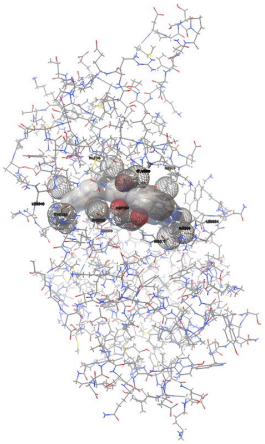
Group	Propensity
small	0.758
hydrophobic	0.95
negative	1.045
positive	1.327
polar	1.098
aromatic	1.5

Table 2. Propensity values of amino acid groups

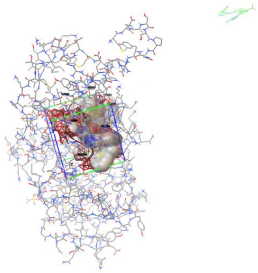
3)

ABL1 gene is a tyrosine kinase, and involved in various cellular processes such as cellular division, differentiation, adhesion, stress response and so on. This gene is considered as protooncogene, meaning that it may lead to cancerous formation when it is mutated. Imatinib, nilotinib, dasatinib are tyrosine kinase inhibitors and lesinurad is URAT inhibitor. [2][3][4][5][6]

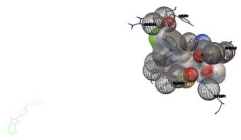
Docking of receptor with Imatinib, Nilotinib and Dasatinib resulted in free binding energy less than -10 kcal/mol. However, docking of receptor with Lesinurad resulted in more than -10 kcal/mol indicating that it may not bind to the receptor as selectively as imatinib, nilotinib and dasatinib. This is expected since imatinib, nilotinib and dasatinib are tyrosine kinase inhibitors whereas lesinurad is a URAT inhibitor.



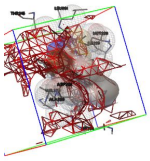
Imatinib analyzed



Nilotinib analyzed



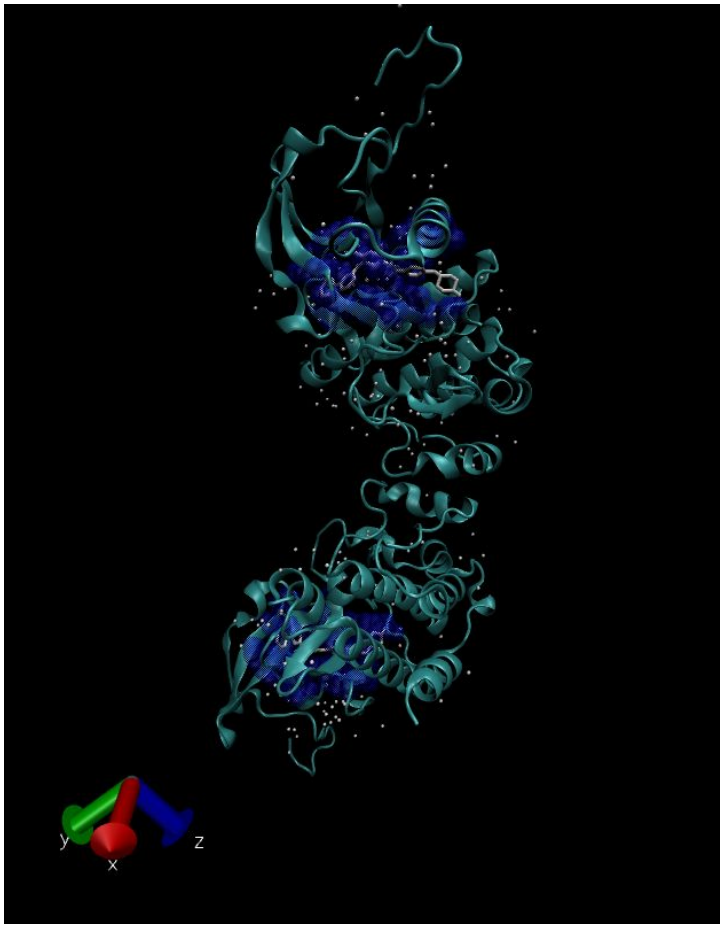
Dasatinib Analyzed



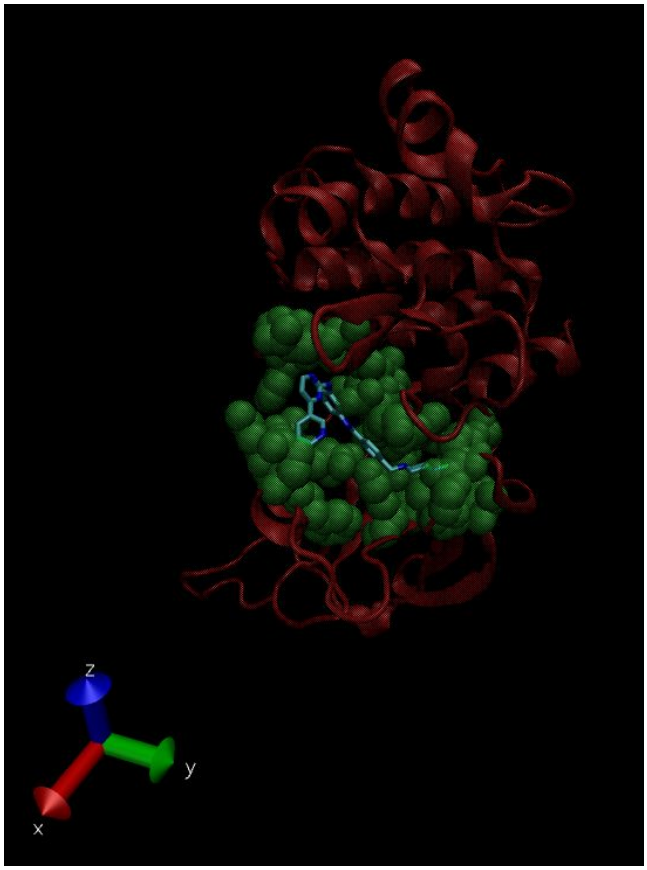
Lesinurad analyzed

One may claim that the cutoff for free binding energy for protein ligand docking is usually below -9 kcal/mol. Based on this cutoff, [7]

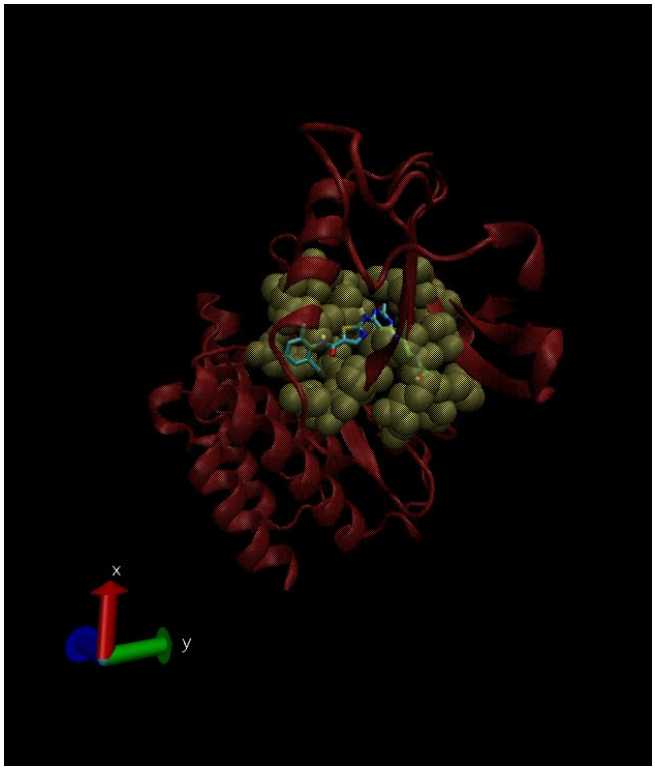
i) Dasatinib has 6 out of 10 acceptable docking runs
ii) Imatinib has 10 out of 10 acceptable docking runs
iii) Nilotinib has 10 out of 10 acceptable docking runs
iv) Lesinurad has 0 out of 10 acceptable docking runs.

b) 

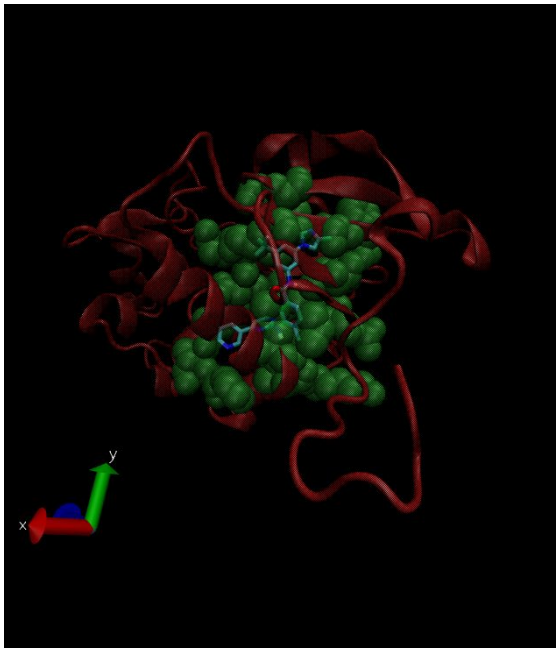
1eip

c) 

imatinib



dasatinib



nilotinib

Lesinurad

In experimental result of imatinib binding the receptor seems different than docking result in a way that the docking result resulted in lower free binding energy. Since the docking performed is called rigid docking, the snapshot of the protein is docked to the ligand. In reality, the residues of protein are more flexible, moving in Brownian motion, which may increase the uncertainty of the protein's binding pattern.

The interacting surface is shown in vDW, the overall protein is shown in newcartoon and the ligand is shown in licorice representation.

Rigid receptor approach cannot include all binding patterns as a well known example; induced fit models of enzymes and their substrates. When enzyme binds to its substrate, they both change in terms of conformation resulting in a better fit. The induced fit model represents the natural process better as opposed to the key-lock models. In our case the rigid docking model resembles the key-lock model more. For that reason flexible docking may be used to improve the docking results.

REFERENCES

[1] Simmerman HK, Kobayashi YM, Autry JM, Jones LR. A leucine zipper stabilizes the pentameric membrane domain of phospholamban and forms a coiled-coil pore structure. J Biol Chem. 1996;271(10):5941-5946. doi:10.1074/jbc.271.10.5941
[2] Database, Gene. “ABL1 Gene (Protein Coding).” GeneCards, www.genecards.org/cgi-bin/carddisp.pl?gene=ABL1
[3] “Imatinib.” DrugBank, www.drugbank.ca/drugs/DB00619.
[4]“Nilotinib.” *DrugBank*, www.drugbank.ca/drugs/DB04868.
[5] “Dasatinib.” DrugBank, www.drugbank.ca/drugs/DB01254.
[6] “Lesinurad.” *DrugBank*, www.drugbank.ca/drugs/DB11560.
[7] Mani, Shankar, et al. Is There Any Borderline of Binding Energy in Docking Stated That We Should Consider These Ligands with Its Binding Energy for Further Analysis? 8 Feb. 2018, www.researchgate.net/post/Is_there_any_borderline_of_binding_energy_in_docking_stated_that_we_should_consider_these_ligands_with_its_binding_energy_for_further_analysis.