FNU Sheenam
Lab rotation Project
Spring 2019 rotation mentor: Prof. Emilio Gallicchio

Title: Binding free energy of posaconazole in two protonation states to a model of Captisol

Aim: To calculate the free binding energy of the drug Posaconazole to Captisol in different protonated states to understand the pH dependence of the observed solubility of posaconazole in the presence of Captisol.

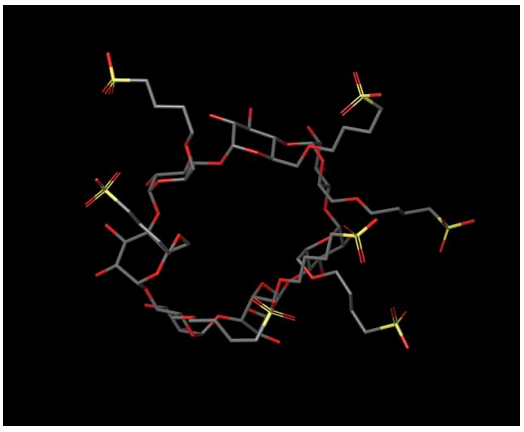
INTRODUCTION

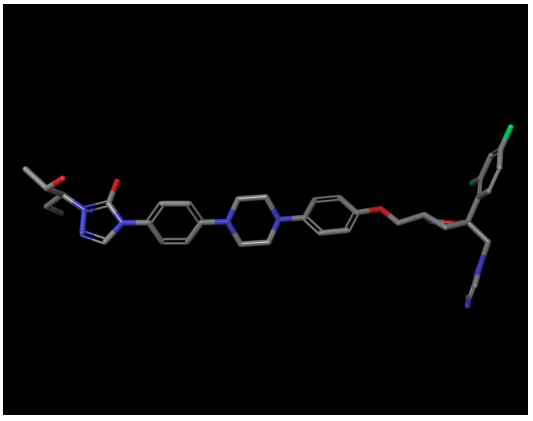
Poor drug solubility is one of the main obstacles in the drug discovery and development process and was recently identified to be strongly related to the choice of target explored. Solubility is the driving force for absorption and acceptable solubility in the intestinal fluid is a prerequisite for achieving sufficiently high drug blood concentrations to obtain a therapeutic effect when systemic effects are warranted.

Essentially, calculating solubility from explicit MD simulations amounts to determining when the chemical potential of the solute in solution is equal to the chemical potential of the solute in its solid, crystalline form (Paluch et al., 2015). This can most readily be achieved by running a series of free-energy calculations, but the chemical potential of the solid is still quite complicated to calculate, since it requires calculation of the fugacity of the solid (Liu et al., 2016).

Posaconazole is a triazole antifungal drug that is used to treat invasive infections by Candida species and Aspergillus species in severely immunocompromised patients. It is structurally related to Itraconazole and has activity against *Candida* species, *Aspergillus* species, *Cryptococcus neoformans,* the zygomycetes and other filamentous fungi.

Posaconazole is a weakly basic and poorly aqueous soluble drug that has poor bioavailability and variable absorption (Heimbecher et al., 2016). Hence, for a better intravenous administration of the drug under aqueous conditions, the drug comes in a composition which consists of a solubilizing agent, a modified β-cyclodextrin such as Captisol, which is the trade name for a sulfobutyl ether-β-cyclodextrin in an acidic solution. On testing different Posaconazole concentrations, under varying Captisol concentrations, it was found out that Captisol solubilizes posaconazole more effectively as the pH of the solution decreases, i.e. under a largely acidic solution. (Heimbecher et al., 2016)





(Above, Left: modified β-cyclodextrin: Captisol; right: Posaconazole)

In an effort to evaluate the range of Posaconazole drug solubilities that could be achieved at feasible pH's, a series of solutions was prepared with a fixed captisol concentration. Utilizing an acidic solution of 20% Capitsol (w/v), the solubility of posaconazole was increased 1000 times (Heimbecher et al., 2016). Thus, we hypothesize that the acidic form of the drug, Posaconazole binds better than the basic form. To test this, we design a computational experiment described below.

METHODS

On MAESTRO, we obtain the protonated forms of the drug Posaconazole by applying a range of pH 7 ± 7 in LigPrep. LigPrep generates accurate, energy minimized 3D molecular structures and optionally expands tautomeric and ionization states from a single input structure.

The two different forms of the posaconazole consisted of the following forms:

1) Unprotonated
2) Singly protonated.

Along with LigPrep for the drug, we exclusively designed the modified β-cyclodextrin molecule, i.e. Captisol on Maestro before heading to the next step of the process.

Once the protonated structures were obtained in MAESTRO, the BEDAM WORKFLOW, which is the Binding Energy Distribution Analysis Method (Gallicchio E. et al, 2010), is an absolute binding free energy estimation and analysis methodology based on a statistical mechanics theory of molecular association and efficient computational strategies built upon parallel Hamiltonian replica exchange, implicit solvation and multi-state statistical inference.

We use the Asynchronous replica exchange simulations (software named ASyncRE) (Gallicchio E. et al, 2016), which allows molecular dynamics threads to progress at different rates and enables parameter exchanges among arbitrary sets of replicas independently from other replicas, for the posaconazole-ligand complexes were run,

we expected the binding energy of the drug to increase from the unprotonated to doubly protonated states.

COMPUTATIONAL SETTINGS

Replica exchange molecular dynamics simulations were conducted for approximately 2 ns per replica with a 1fs time-step with exchanges every 50 ps. Binding energy samples were collected every 10 ps. We employed 16 replicas ranging from λ values 0 to 1, which is a dynamic variable in free energy simulations and controls the progress of different interaction, thereby simultaneously evaluating thermodynamic properties for multiple states in a single simulation. The total simulation time for each simulation was 32 ns.

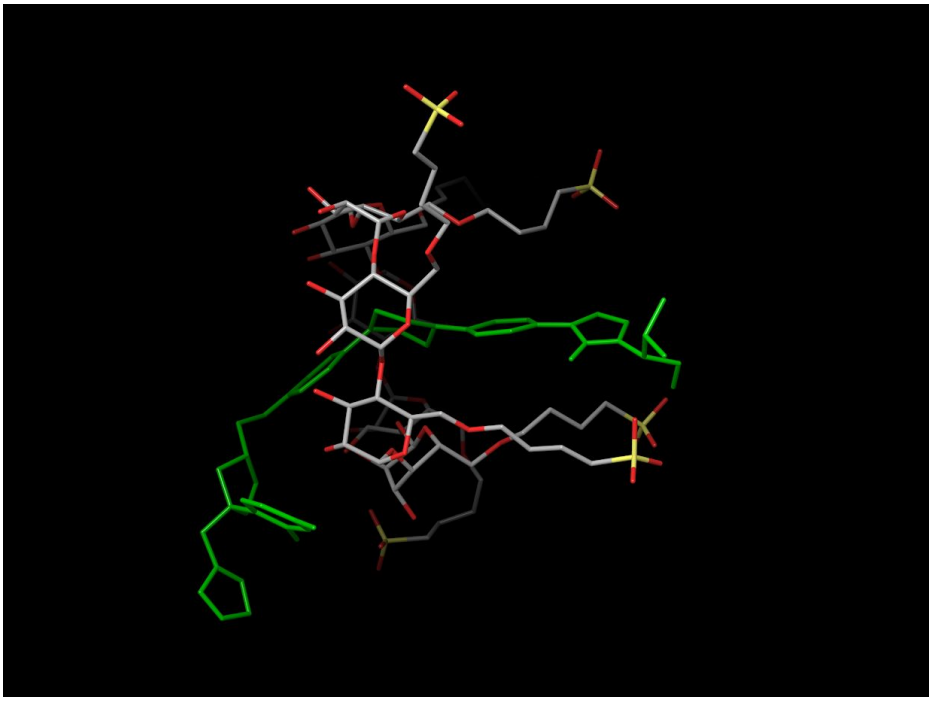
RESULTS & DISCUSSION

On obtaining the binding free energies of the protonated posaconazole states vs single protonated states with Captisol, it was found out that unprotonated posaconazole-captisol complex had the lesser negative binding free energy than the protonated posaconazole-captisol complex by the order of about 5 kJ/mol.

The results of Asynchronous replica exchange simulations for both Complex-1 and Complex-2 were obtained as follows:

Complex	Total charge on Posaconazole	DGb (Binding Free Energy) (kCal/mol)	DE (Average Interaction Energy) (kCal/mol)
Complex-1 (Unprotonated posaconazole-captisol complex)	0	-4. 90	-25.11
Complex-2 (Singly protonated posaconazole-captisol complex)	+1	-10.21	-39.53

Structural Analysis of interactions in Complex-1 and Complex-2

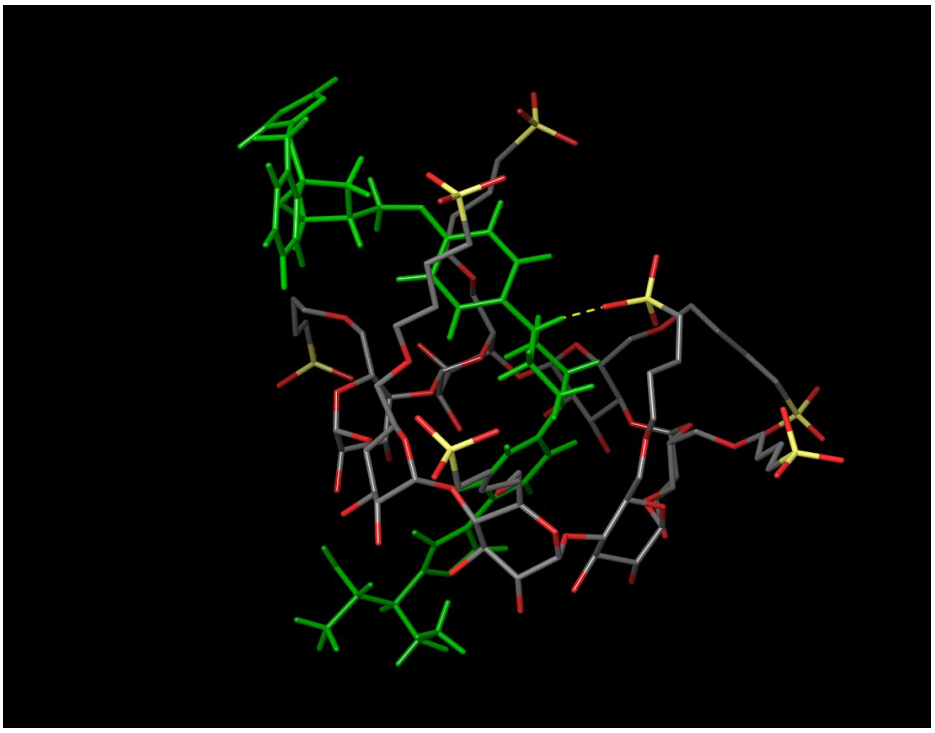


(Above: Unprotonated posaconazole-Captisol complex at λ=1)

The posaconazole molecule assumingly fits itself into the cavity of the modified B-cyclodextrin, thereby trying to maximize interactions with the different moieties on the captisol molecule (charged and uncharged).

The picture obtained above is derived at the λ=1, where the interaction between the drug and the ligand is maximum, thereby minimizing repulsions or any other non interacting physical parameters.

As can be seen, the uncharged or the unprotonated posaconazole drug molecule is unable to establish any other strong interactions with ligand, such as Hydrogen bonding, dipole-dipole interactions or formation of salt bridge due to strong ionic interactions.



(Above : Singly protonated posaconazole-Captisol complex at λ=1)

However, in the case of complex-2, a singly protonated posaconazole molecule, carrying a +1 overall charge, at one of the susceptible N atoms on the chain under moderately acidic environment, maximizes its interactions with one of the seven sulfate moieties lying on the exterior of the captisol ring.

This results in the formation of a salt bridge, thereby leading to a higher and much stronger interaction than what was in the case of complex-1, leading to a higher negative free binding energy and average interaction energy.

CONCLUSION

Posaconazole solubility increases in an acidic medium of a fixed concentration of Captisol. The results of the computational experiments conducted here confirm the hypothesis that this effect is due to the higher affinity of the protonated form of Posaconazole relative to the unprotonated one.

The positive charge on the drug, i.e. the protonation site provides a good hotspot for strong ionic interactions with the sulfate groups lying on the exterior of the captisol, something that is not quite present in the neutral Posaconazole form when complexed with the same Posaconazole molecule.

For future studies, one can interestingly also see the interaction of a doubly protonated posaconazole-ligand complex to check how two positively charged moieties in the drug can possibly interact with the negative groups of captisol, and observe if the binding free energies are more negative.

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