# Biophysical Investigation of Tyrosine Kinase Inhibitors as Ligands of G-quadruplexes

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## Abstract

Telomeres are sequences of repeating nucleotides located at the ends of chromosomes. Telomerase is an important enzyme present in human cells that can elongate telomeres by adding nucleotides, protecting DNA from degradation during replication. Telomerase is overexpressed in 85% of cancer cells and absent in normal human somatic cells, and contributes to uncontrollable replication of cancer cells. Human telomerase reverse transcriptase (hTert) has been shown to be integral to the functionality of telomerase in cancer cells. Furthermore, disruption of hTert activity leads to crisis and cell death. Currently there are no FDA approved drugs that target telomerase activity. Recently, our labs have identified a variety of FDA approved tyrosine kinase inhibitors (TKIs) that bind to both telomeric and promoter DNA structures known as G-quadruplexes (G4s). G4s are stable secondary DNA structures composed of stacked guanine quartets. Thus, this research project utilizes molecular dynamics (MD) simulations to investigate the interactions between these quadruplexes and TKIs, as well as classical biophysical approaches to investigate four TKIs and their interaction with both telomeric and hTert core promoter G-quadruplex structures. In doing so, this research provides valuable insight into a drug class that could be modified to selectively target cancer cells. This research was supported by the NCI R25 University of Louisville Cancer Education Program (R25- CA134283)

## Methods

- Molecular dynamics simulations provide valuable insight into the movement and interactions between molecules by numerically solving Newton's equations. The AMBER software package was utilized to conduct molecular dynamics simulations in this research project. Sunitinib, Imatinib, Osimertinib, and Ponatinib and their interaction with telomeric and hTert core promoter G-quadruplex structures were analyzed in this project. First, each was docked to the receptor (either hTert or Tel48) using Glide based on stoichiometries determined through analytical ultracentrifugation. The systems were prepared and explicitly solvated in a solution containing water molecules and potassium ions to neutralize the charge of the system. The systems were then heated and equilibrated. Next, 20ns molecular dynamics simulations were run on a total of eight systems consisting of the four ligands, each docked to both hTert and the telomeric structure. Additionally, change in binding free energy was calculated for each system (taking into account solvent) to determine ligand binding affinities and the most stabilizing attachment sites in the complexes. Shown below are the methods of calculating the binding free energy:

$$\Delta G^0_{bind,\ solv} = \Delta G^0_{bind,\ vacuum} + \Delta G^0_{solv,\ complex} - (\Delta G^0_{solv,\ ligand} + \Delta G^0_{solv,\ receptor})$$

- Circular Dichroism (CD): Spectra were obtained in BPEK (200mM KCl, 1mM EDTA, 8mM $PO_4^{2-}$, pH 7.2) at 20.0˚ C in a JASCO-710 spectropolarimeter using oligos purchased from IDT and annealed at 99.9˚ C for 20 minutes before slow cooling. Parameters were 20.0˚ C cell temperature, scan range of 500nm (or less) to 240nm, 1nm Pitch, 4s response, and averaging of 4 to 10 scans. Spectra were normalized to concentration using the following formula: $\Delta\varepsilon = mdeg/(32{,}980*C*L)$.
- Analytical Ultracentrifugation (AUC): Sedimentation velocity experiments were carried out on a Beckman Coulter ProteomeLab XL-A centrifuge in BPEK buffer at 20.0˚ C with a run speed of 35k rpm in continuous mode using standard 2-sector cells. Analysis was performed in Sedfit using a continuous distribution C(s) model (non-interacting species) with 100 scans using wavelengths appropriate for each species (260nm for DNA, 310-360nm for drugs).

## Acknowledgments

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## MD Simulation Analysis

### Front and back views of the four hTert systems at the end of simulation

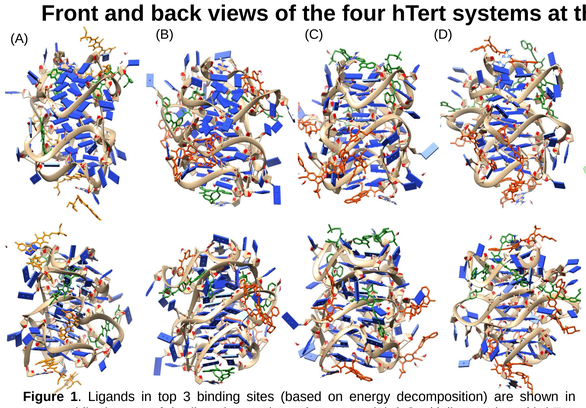



**Figure 1**. Ligands in top 3 binding sites (based on energy decomposition) are shown in green, while the rest of the ligands are shown in orange. (A) 8 Sunitinib complex with hTert (B) 8 Imatinib complex with hTert (C) 9 Osimertinib complex with hTert (D) 9 Ponatinib complex with hTert

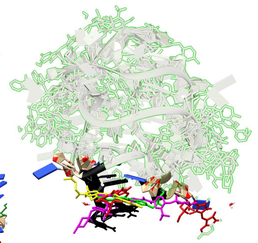


**Figure 2.** All 4 hTert systems overlaid after simulation. Black residues are most stabilizing, and each ligand is shown in a separate color bound in the 3' end.

### Front and back views of the four tel48 systems at the end of the simulation

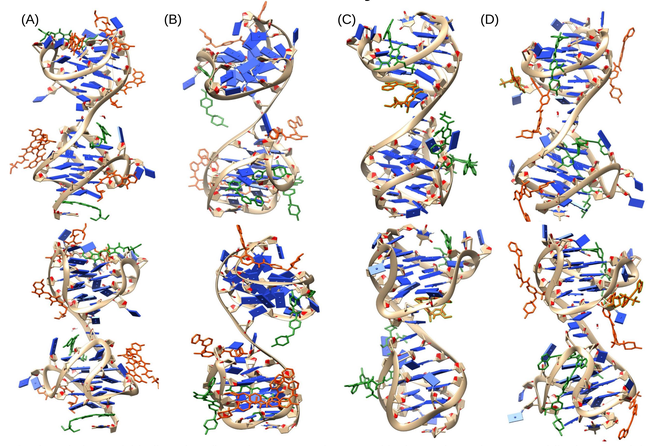



**Figure 3**. Ligands in top 3 binding sites (based on energy decomposition) are shown in green, while the rest of the ligands are shown in orange. (A) 9 Sunitinib complex with tel48 (B) 7 Imatinib complex with tel48 (C) 4 Osimertinib complex with tel48 (D) 7 Ponatinib complex with tel48

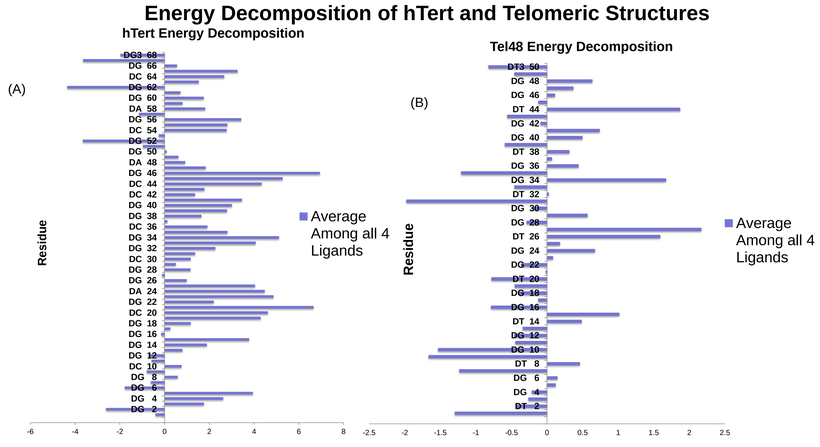
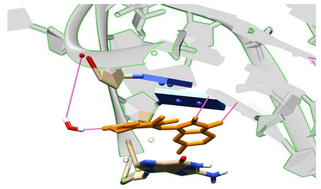



**Figure 4.** (A) Energy decomposition of hTert residues. (B) Energy decomposition of tel48 residues

| Complex | Ligand | Initial Ligands | Final Ligands | MMGBSA ΔG (kcal/mol) | MMPBSA ΔG (kcal/mol) | AUC Stoichiometry | ΔTm (˚ C) | Notes |
|---|---|---|---|---|---|---|---|---|
| Tel48 | Sunitinib | 10 | 9 | -177.9525 | -202.0304 | 12 | 21.7 | 1 ligand falls off |
| | Imatinib | 7 | 7 | -97.0399 | -132.7711 | 7 | 13.4 | all ligands attached |
| | Osimertinib | 4 | 4 | -66.7323 | -95.2199 | 4 | 16.6 | all ligands attached |
| | Ponatinib | 7 | 7 | -145.5959 | -166.6784 | 7 | 0.3 | all ligands attached |
| hTert | Sunitinib | 9 | 8 | -124.7949 | -144.8371 | 7 | 9.8 | 1 ligand falls off |
| | Imatinib | 8 | 8 | -72.6796 | -125.2823 | 9 | 3.8 | all ligands attached |
| | Osimertinib | 9 | 9 | -102.965 | -164.1218 | 4 | 4.7 | all ligands attached |
| | Ponatinib | 9 | 9 | -159.9351 | -207.4336 | 7 | 6.9 | all ligands attached |

**Figure 5:** Table of MD simulation results, Generalized Born and Poisson-Boltzmann binding energy calculations, AUC stoichiometries, and Tm shifts for each ligand and corresponding complex



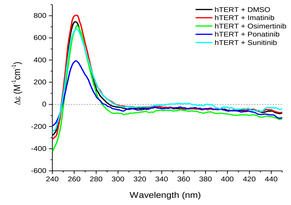



**Figure 6.** Hydrogen bonding (shown in pink) of Sunitinib in 3' site of hTert

**Figure 7.** CD spectra of hTert, as well as hTert attached to each ligand.




**Figure 8.** Results of hTert + Imatinib AUC. The shift in sedimentation of hTert when bound to Imatinib shows increased molecular weight (proportional to the sedimentation coefficient) due to binding of the ligands. Integration of the ligand peaks shows change in free ligand concentration, allowing us to calculate the stoichiometry of bound ligands




**Figure 9.** CD spectra of tel48, as well as tel48 attached to each ligand. Possible induced CD is shown for ponatinib and sunitinib, suggesting end-pasting.

## Conclusions

Although there are extensive studies on drug:G4 interactions, there is little research on structures as large as tel48 and hTert, which contain sets of 2 and 3 quadruplexes respectively. By analyzing the ligand energy decomposition within the complexes, we found that the endpoints of both hTert and the telomeric structure are likely the preferred drug binding sites. In complex with hTert, all four ligands were found bound to the 3' end. The 5' site of hTert is a bit less stabilizing, with only two of the four ligands binding. In three of four telomeric systems, ligands were bound to the 5' end, one of the more stabilizing sites based on the free energy decomposition. The energy decomposition analysis also showed residues 31 and 35, a thymine and guanine in a groove of the telomeric system, as two of the most stabilizing residues. In accordance with this observation, the simulations showed three of the four ligands bound in the corresponding site. Importantly, this site may aid future virtual drug screening campaigns aimed at selectively targeting telomeres. CD spectra of hTert and the ligands showed strong indication of hTert-Osimertinib interaction by decreases at 240 and 260nmwhile Sunitinib and Imatinib showed only weak shifts in the spectrum and no induced CD (ICD). CD spectra of tel48 and each ligand shows possible induced CD for Sunitinib and Ponatinib, which indicates an end-pasting mechanism. The MD simulations provide valuable theoretical data and show that a set number of specific ligands can be bound to these complexes, and even show probable sites. In future work, the stoichiometries and binding free energies will be verified experimentally (i.e. via isothermal titration calorimetry) to more accurately measure free energy and stoichiometry to validate the MD analyses.