Decoding the Conformational Plasticity of Ternary Complexes for Protacs' Differential Activity Based on Long Term MD Simulation and QM Based Studies

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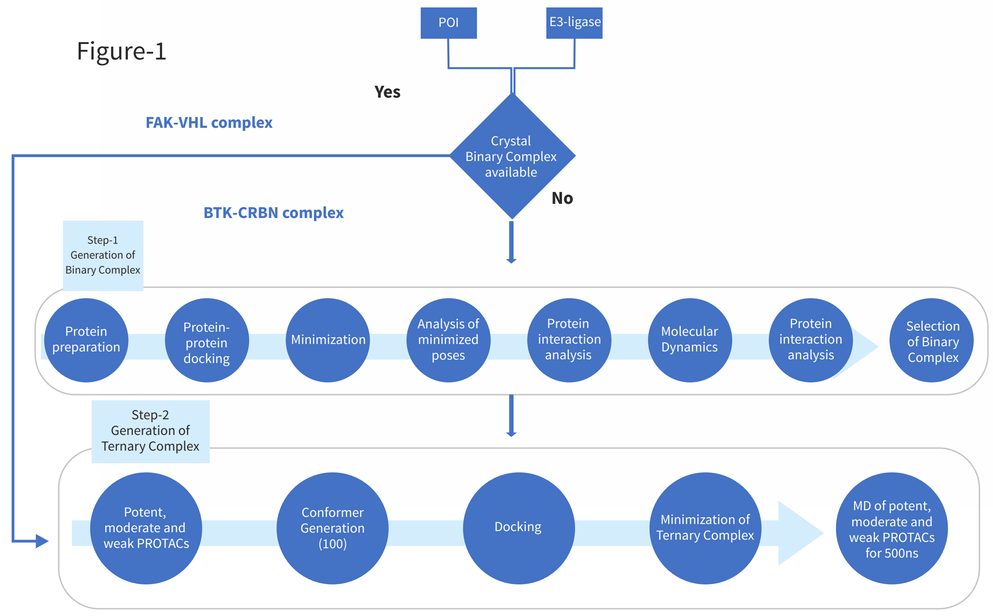
Abstract

- Targeted protein degradation by proteolysis-targeting chimeras (PROTACs) is a new modality to target undruggable targets.
- Mechanism of PROTAC-induced degradation is dependent on formation of proper ternary complex which mainly depends on the plasticity of ternary complex.
- Utilizing long term MD simulations (500ns), current work provides insights into the role of plasticity of ternary complexes with differential activity. We took two ternary complexes (FAK-VHL & BTK-CRBN complexes) to understand the phenomenon.
- Furthermore, we utilized quantum mechanical (QM) calculations to understand the differential stability of PROTAC-mediated ternary complexes. This report provides deeper insights into the molecular mechanism of PROTACS's differential activity for therapeutic intervention.

Methods

Figure-1



- Kinase POI (FAK & BTK) were taken for the present study based on:
  a) availability range of activity values
  b) availability of crystal structure
- The binary complex of FAK with E3-ligase-VHL- was available with PROTAC (PDB ID:7PI4) in the PDB.
- The BTK-CBN binary complex was built using PIPER module of Schrodinger.
- Both these complexes were taken as template for the present study.
- The ligands (potent, moderate, and weak PROTACs) used to build the ternary complexes were taken from the literature and minimized using OPLS4 FF in GLIDE module of Schrodinger and were subjected to MD simulation.
- Workflow of computational methodology given in Figure-1.

Results

- In FAK-VHL complex it was observed that the interacting residues between the POI and the E3-ligase (Cys427 in FAK and Arg69 in VHL) is present in potent PROTAC throughout the 500ns MD simulation. These interactions were intermittent and was absent in moderate or weak PROTACS (Figure 2A&2B).
- Docking of the PROTACS in the binary complex showed that the potent PROTAC binds to the complex in proper orientation which is not shown by the weak ones (Figure-2C&2D). Overlay of the MD poses, exhibited the dynamic behavior of the loops around E3 ligase binding site (Figure 2E). These dynamic behavior of the loops impacts the differential binding of the PROTACS-leading to differential potencies.
- QM calculations of Hydrogen bond strength energy and vibration frequencies (H-bond stretching strength) for potent and weak ternary complexes at E3 ligase domain were calculated using Jaguar DFT module from Schrodinger. (Figure-3A & 3B)
- QM calculations in the VHL binding domain to PROTACS reveals differential binding w.r.t their potencies. For potent PROTACS, the HUMO-LUMO gap observed was 0.081 kcal/mol (Figure-3A&3B) whereas it was 0.065 kcal/mol for the weak PROTACS. Additionally, H-Bond stretching energy (HSE) and Vibrational frequency (VF) of potent and weak PROTACS were also calculated at DFT level QM. We observed that for potent PROTAC, HSE was -4.41 kcal/mol and VF of complex was -3984.07 Hz whereas for weak PROTAC HSE was -3.82 kcal/mol and VF of complex was 3862.66 Hz (Figure-3C&3D)
- Similar observation were also seen in BTK-CRBN complexes between the ternary complexes potent and the weak PROTACS.

Figure-2A(Potent)

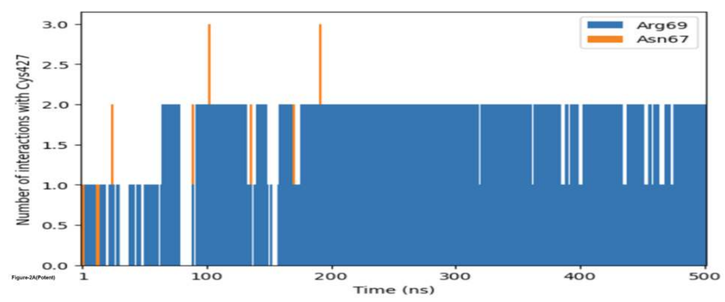
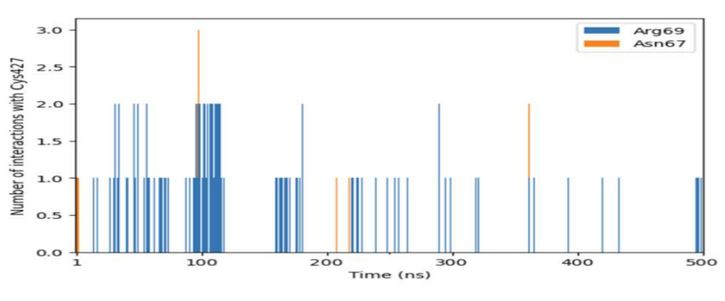


Figure-2B (Weak)



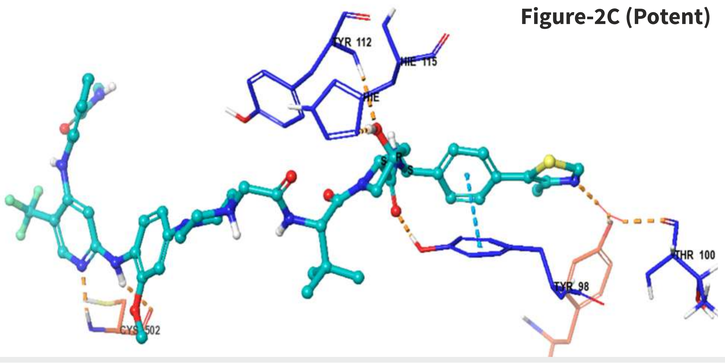


Figure-2C (Potent)

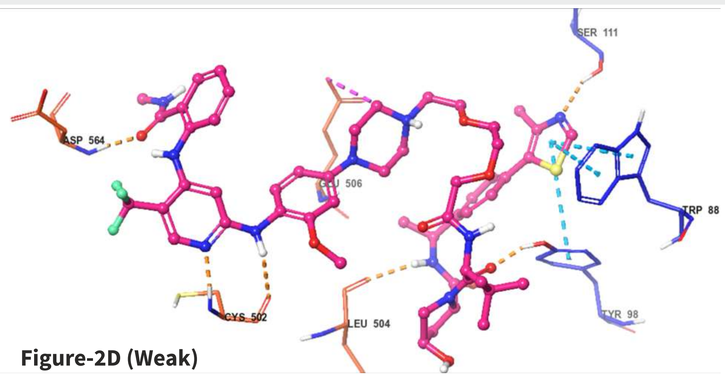


Figure-2D (Weak)

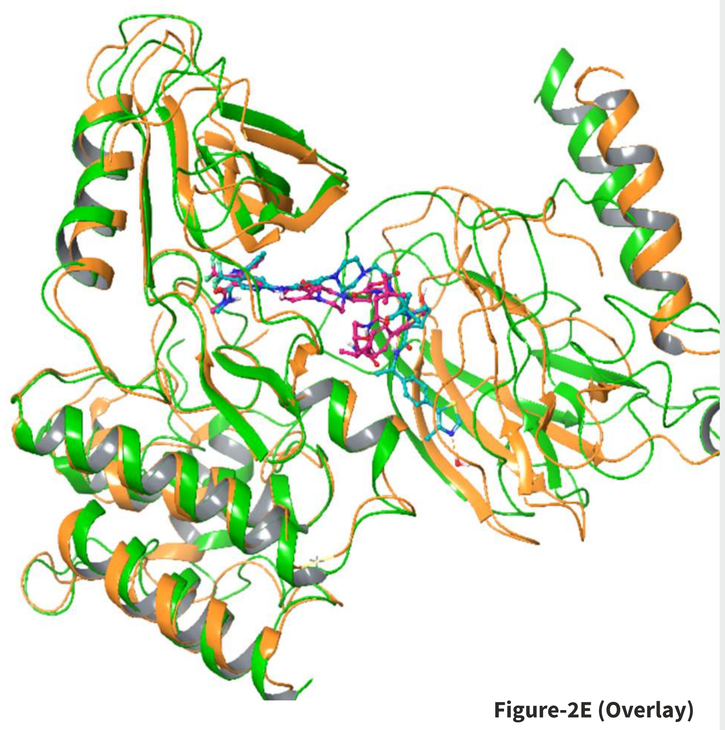


Figure-2E (Overlay)

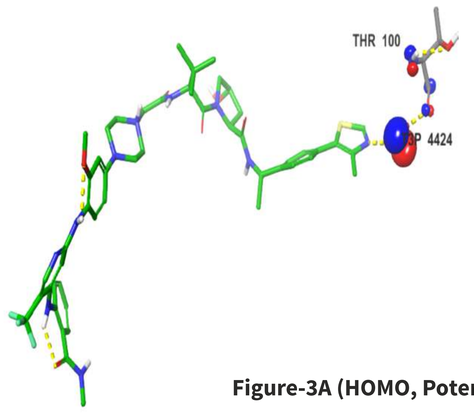


Figure-3A (HOMO, Potent)

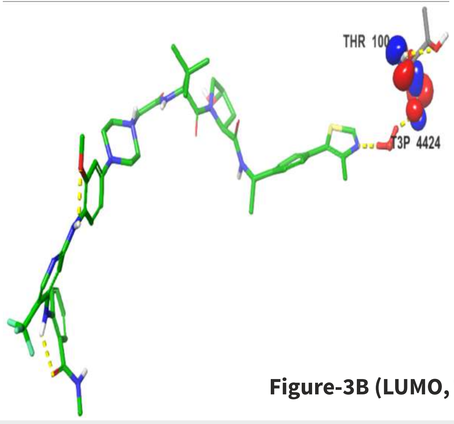


Figure-3B (LUMO, Weak)

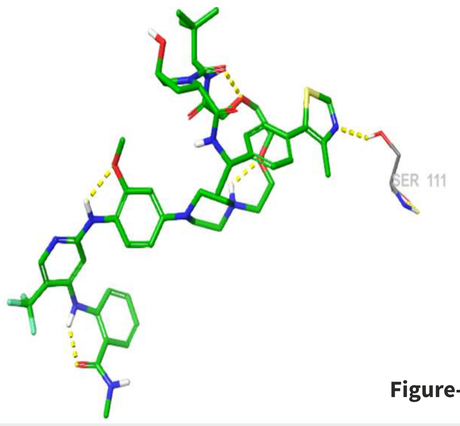


Figure-3C

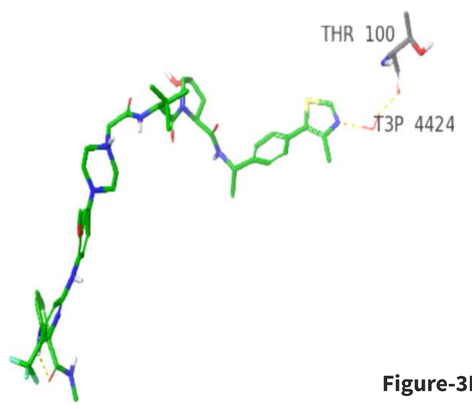


Figure-3D

Conclusions

- Our present study provides deeper insights into the molecular mechanism of PROTACS differential activity for therapeutic intervention
- The dynamic nature of the binary complex determines the feasibility of PROTACS binding and formation of viable ternary complex
- The strength of H-bond formation in the E3-ligase determines the formation of ternary complex differentiating their activities
- Our present protocol is able to identify the molecular mechanism of PROTACS activity even when the crystal structure of the binary complex is not available