From Binding to Degradation : Multiscale Insight into PROTAC-Mediated Protein Targets

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Abstract

- Mechanism of PROTAC-induced degradation is dependent on the plasticity of ternary complex.
- MD simulations (500ns each) for three PROTAC complexes (FAK-VHL & BTK-CRBN & TTK-CRBN), provided insights into the role of plasticity of ternary complexes with differential activity.
- Simulation events analysis revealed that potent PROTACs maintained stable interactions between the protein of interest (POI) and the E3 ligase, in contrast to the weaker PROTACs.
- Distributions of the free energy landscape offered newer insights into the stability of population states for understanding the degradation potential (Figure-1).
- We highlight the importance of QM and DFT based methods to predict the impact of binding at the E3-ligase site which corelates with the degradation potentials of PROTACs.

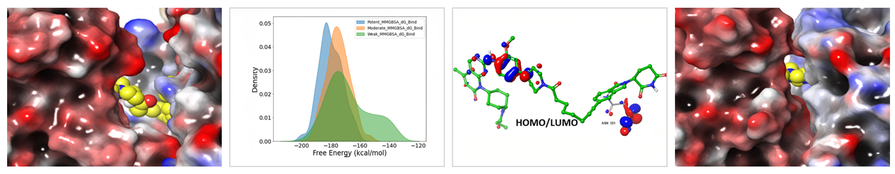


Figure-1

Methodology

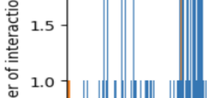
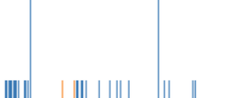
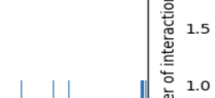
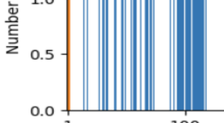
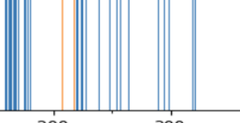
FAK-VHL	BTK-CRBN	TTK-CRBN
 Potent-PROTAC (DC_{50} = 1.3 nM)	 Potent PROTAC (DC_{50} = 0.5 ± 0.2 nM)	 Potent PROTAC (DC_{50} = 1.5nM)
 Moderate PROTAC (DC_{50} = 31.62nM)	 Moderate PROTAC (DC_{50} = 31.2 ± 16.4 nM)	 Moderate PROTAC (DC_{50} = 46 nM)
 Weak PROTAC(DC_{50} = 158.48nM)	 Weak PROTAC (DC_{50} = 113 ± 20 nM)	 Weak PROTAC (DC_{50} = 598.7 nM)

Figure-2: PROTACs used for molecular docking and dynamics

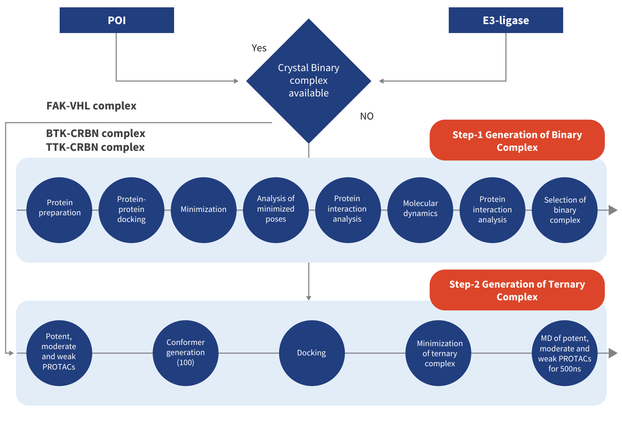


Figure-3: Computational Workflow for PROTAC modelling

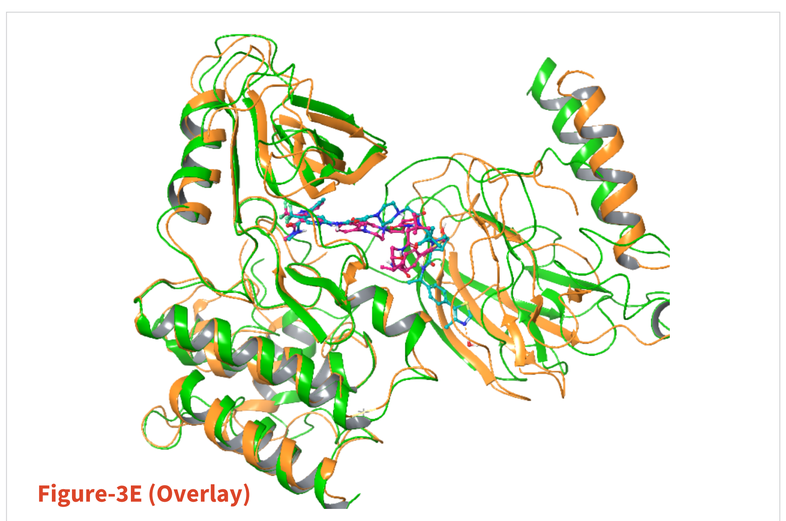
- Protein Kinases (FAK, BTK & TTK) were taken based on availability of activity values & crystal structures
- Ternary complex of FAK and E3-ligase-VHL is available in the protein data bank (PDB ID:7PI4).
- For BTK-CRBN & TTK-CRBN, we developed in-house the binary) complexes.
- PROTACs (potent, moderate, and weak PROTACs) used to build the ternary complexes were taken from the literature and subjected to docking and MD simulation (Figure-2 & 3).
- QM and Free energy landscape was calculated using the modules from Schrödinger discovery suite.

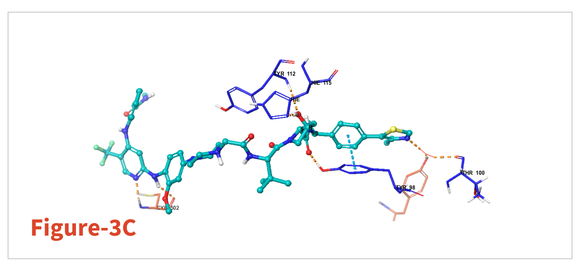
Results and Discussion

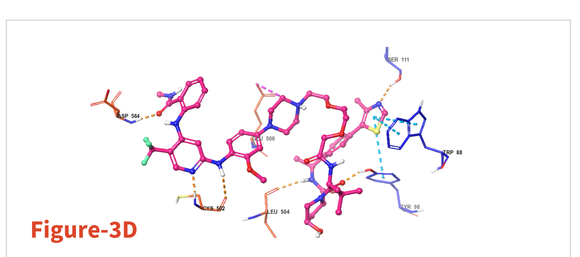
- In FAK-VHL complex it was observed that crucial Protein-Protein interacting residues between the POI and the E3-ligase (Cys427 in FAK and Arg69 in VHL) is present in potent PROTAC throughout the 500ns MD simulation whereas in moderate or weak PROTACS these interactions were either less in number or completely absent (Figure 3A & 3B).
- 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-3C & 3D). Overlay of the MD poses, exhibited the dynamic behavior of the loops around E3 ligase binding site (Figure 3E). 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 crucial residue are calculated using Jaguar DFT module from Schrodinger. (Figure-4A & 4B)
- QM calculation in the in the VHL binding to PROTACS reveals differential binding of the PROTACS are described in Table 1 & H-bond of potent and weak PROTACs are shown in Figure-4C & 4D.

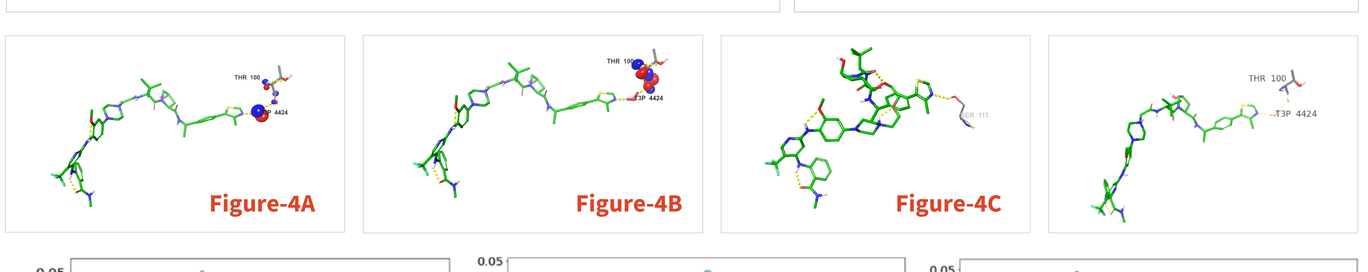
QM/DFT results	Potent-PROTAC	Weak-PROTAC
HSE (Kcal/mol)	Thiazole-N – HOH = -3.35	Thiazole-N – Ser111 = -3.82
	HOH-Thr100 = -1.06	
HOMO (eV)	-0.25	-0.25
LUMO (eV)	-0.17	-0.19
H-L (eV)	0.08	0.07
VF (Hz)	3984.08	3862.66

Table-1: QM/DFT calculation of potent and weak PROTACs









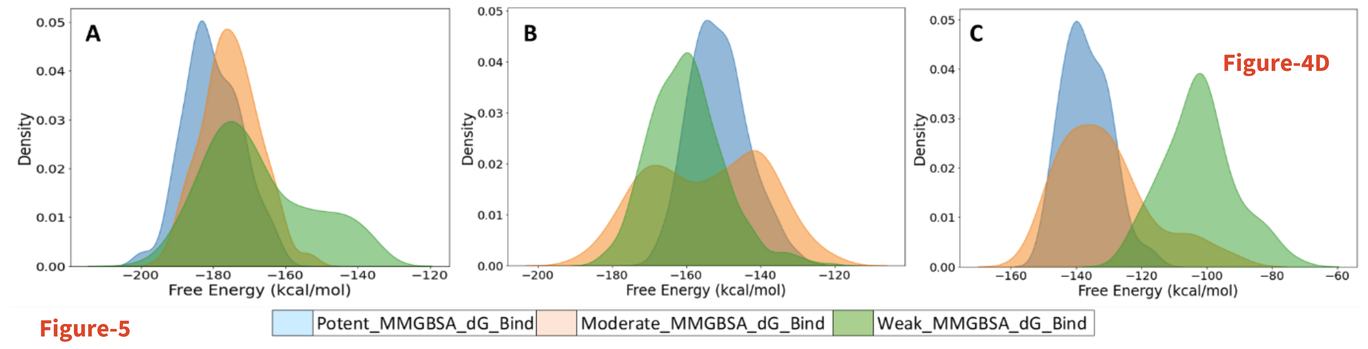


Figure-5

Conclusions

- Using a multiscale approach (ternary complexes' conformational plasticity along with their free energy landscapes and the strength of binding of E3-ligase binders) our present study opens new avenues for design of PROTACs for efficient degradation.
- The strength of H-bond formation in the E3-ligase along with the dynamic nature of the binary complex determines the strength of formation of ternary complex.
- Our present protocol provides a novel integrated framework to determine the molecular mechanism of PROTACS activity even in absence of the crystal structure.