

**Exploring the thermodynamic, kinetic and inhibitory mechanisms of  
5-iTU targeting mitotic kinase haspin by integrated molecular  
dynamics**

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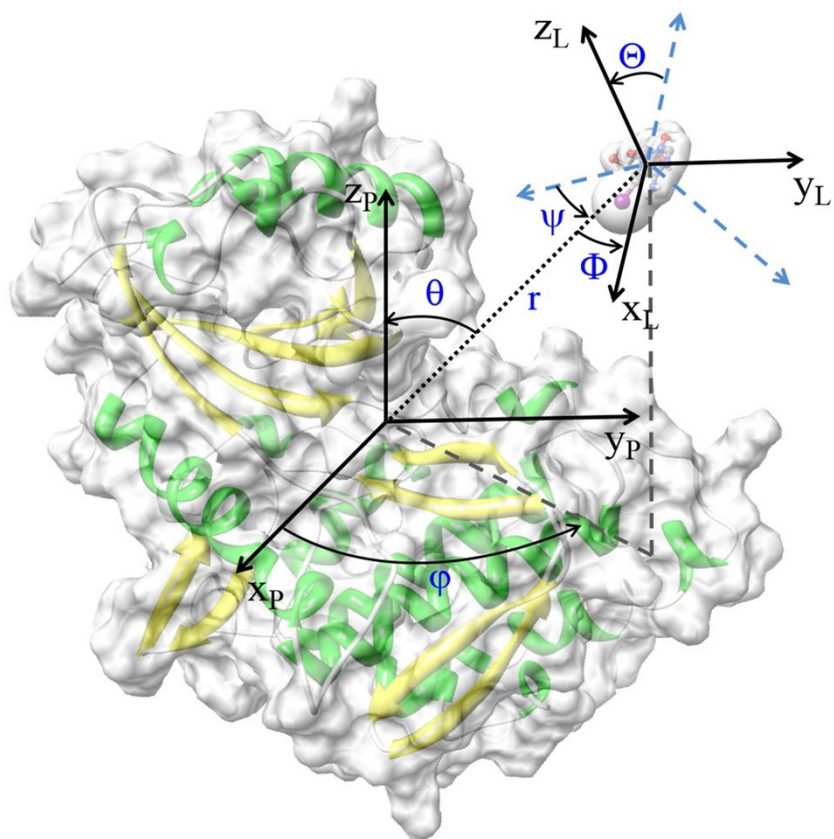
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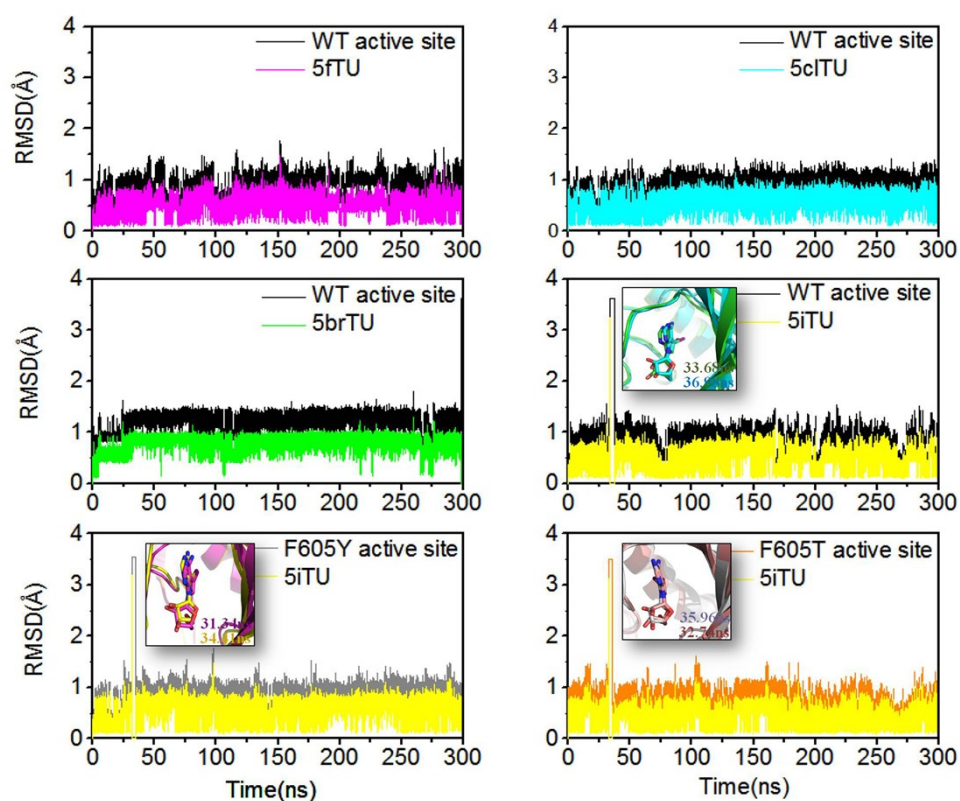
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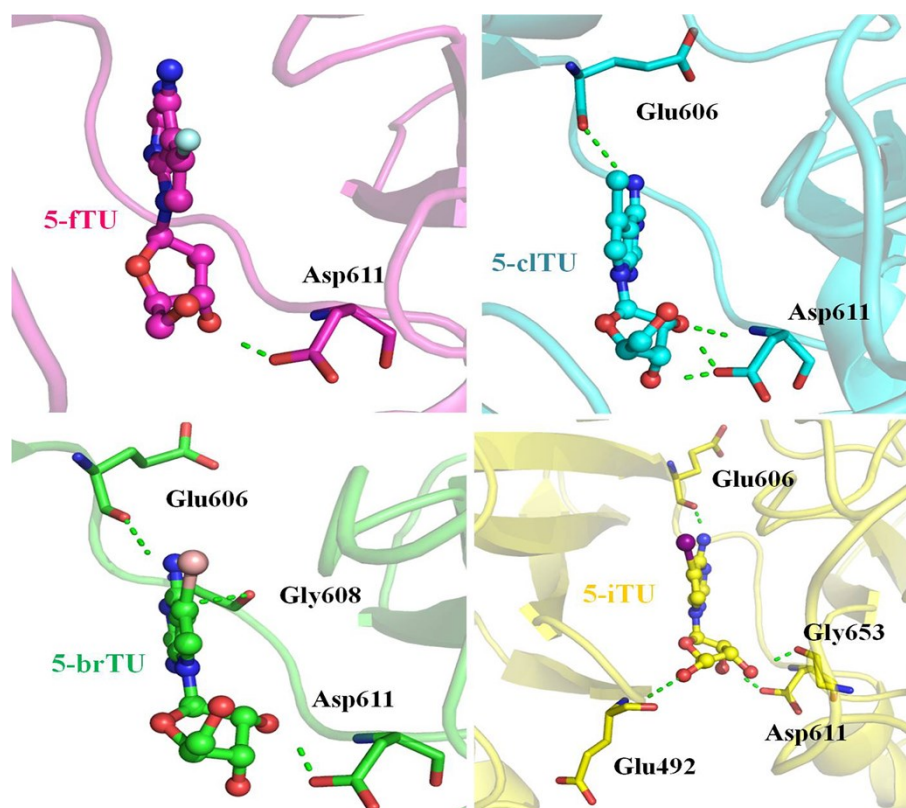
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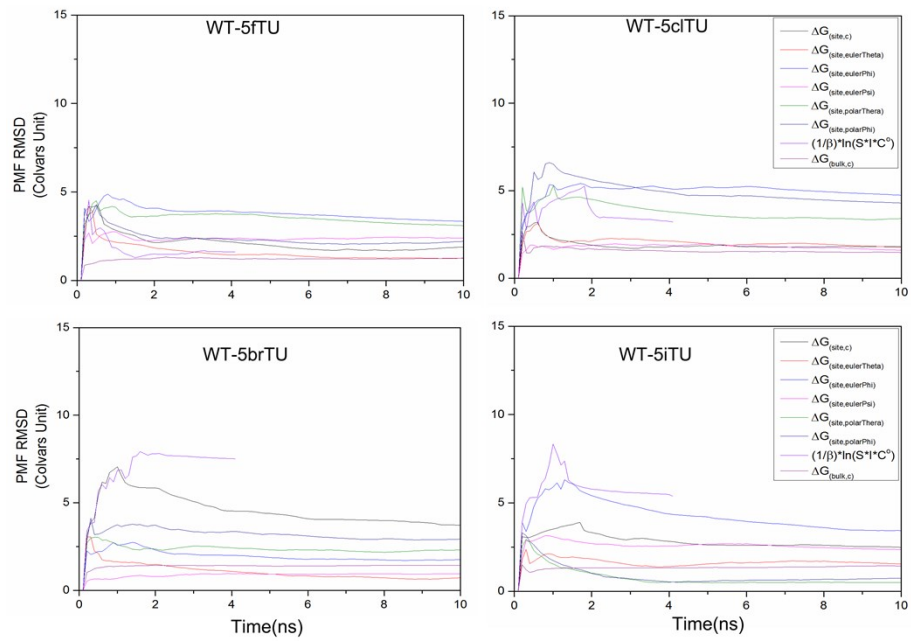
**Figure S1.** Degree of freedom considered in the standard binding free energy calculation strategy. Euler and spherical angles describe the relative orientation and position of inhibitors with respect to haspin, respectively.



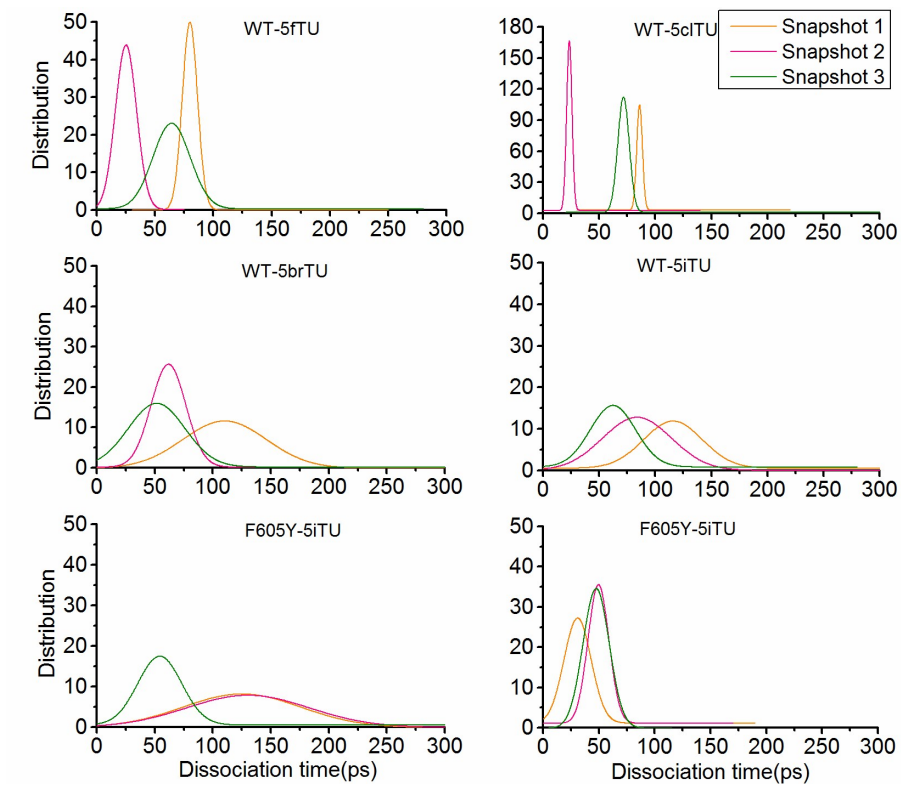
**Figure S2.** Time series of RMSDs of 5-iTU derivatives and WT/F605Y/F605T haspin active site (protein heavy atoms within 5 Å of inhibitor) for each system. Note: the relative significant RMSD variations in 5-iTU and WT/F605Y/F605T haspin complexes were not induced by their structural changes, but the lack coordinate records of random simulations with the common parameter setting “ntwx=1000”. Inset showed that the complexes aligned very well before and after RMSD fluctuations in each system.



**Figure S3.** Binding modes of haspin with 5-iTU and derivatives. The green dashed lines represent the hydrogen bond interactions.



**Figure S4.** Time series of potentials of mean force (PMF) RMSDs averaged for stages in each step for each system.



**Figure S5.** The Gaussian distribution of dissociation time for each snapshot in each system.