Molecular Dynamics-Assisted Interaction Between HABT and PI3K Enzyme: Exploring Metastable States for Promising Cancer Diagnosis Applications

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ABSTRACT

ABSTRACT – The present study investigates the interaction between the fluorescent molecule HABT and the PI3K enzyme, a cancer-related target, under nonequilibrium conditions. Using molecular docking, molecular dynamics simulations, and quantum calculations, the work explores metastable states and their influence on the Excited State Intramolecular Proton Transfer (ESIPT) process. Results show that 97% of HABT conformations in water support ESIPT, while only 63% do so within the protein environment due to competitive intermolecular interactions, showing an increased enol emission in the protein-docked environment. These findings highlight the importance of including metastable states when modeling biological probes, and demonstrate promising fluorescent properties of HABT, supporting its application as a promising diagnostic probe for cancer detection.

Keywords: Biased MD Simulations, ESIPT, fluorescent probes, spectroscopic probes.

Introduction

Biological systems often function in local nonequilibrium regimes, a concept introduced by Schrödinger [1], which considered living systems as open systems. This concept was later applied to key cellular processes such as protein folding and signal transduction. However, one of the main goals of this perspective was its relevance in studying ligand–target interactions in drug discovery [2]. Therefore, given the global impact of cancer and the challenges of current treatments, the usage of such perspective can be an interesting strategy to combat this illness.

In the aim of a better and efficient diagnosis, fluorescent probes, especially those relying on Excited State Intramolecular Proton Transfer (ESIPT), offer high sensitivity and biocompatibility for diagnostic applications. In this scenario, this study focuses on the interaction between the PI3K enzyme, a key cancer target [3], and the HABT molecule, an ESIPT-capable probe [4], using enhanced sampling methods to explore metastable states and their effects on ESIPT efficiency [5]. From this, the present study was able to obtain important insights into a promising cancer diagnosis strategy: the usage of fluorescent probes for biomarkers signaling.

Computational Methods

HABT construction and parametrization, and Molecular Docking.

The HABT structure was constructed using GaussView 6 software, and later optimized with Gaussian 9, using B3LYP functional and 6-311g basis set. The parameters obtention for further MD simulations was performed with the Automated Force Field Topology Builder (ATB) 3.0. With the constructed ligand, Molecular Docking was performed to provide the initial structure for MD simulations. This procedure was performed using AutoDock Vina 1.1.2 software, with the PI3K crystal structure (PDB ID: 3QJZ).

Biased MD Simulations.

To explore metastable states, biased MD simulations were conducted using GROMACS 2021.4 patched with PLUMED 2.8.0. The used biasing technique was the on-the-fly probability enhanced sampling technique (OPES) expanded. As biased CV it was used the potential energy, using as target the ensemble over a temperature range of 300K-600K.

Metastable states analysis and spectroscopic investigation.

To observe the obtained metastable distribution from biased MD simulations PCA was conducted. It was also possible to select representative conformations and perform an investigation of HABT spectroscopy in the simulated environments. For this investigation, TDDFT calculations were conducted using cam-B3LYP functional and TZVP basis set.

Results and Discussions

From the performed investigations, Figure 1 shows how the accessed metastable states were distributed. Figure 1.a shows that only a few conformations with unapproppriate ESIPT geometry were stabilized in the system HABT+Water (around 3% of all conformations). However, Figure 1.b shows that when considering the protein environment, this number increases for 37% of all conformations. Therefore, due to the presence of residues ASP950 and LYS833, much more ESIPT unapproppriate conformations were stabilized.

As further analysis, representative conformations of each accessed metastable state was taken for fluorescent properties investigation. In Figure 1.b, the metastates were divided into two, one at PC2>0 (label A) and the other at PC2<0 (label B). In each metastate, the conformation closer to the center of its respective cluster was selected as a representative conformation of its metastate.

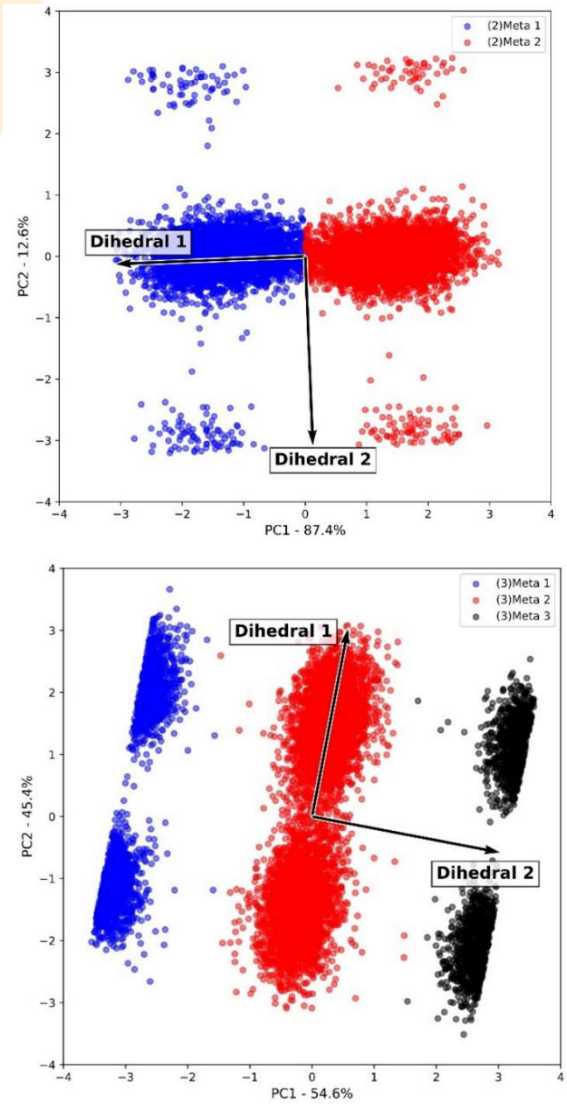

Figure 1. PCA conducted for a) HABT in water only, and b) protein-docked HABT in water reservoir.

Therefore, 6 conformations were selected, being them named as meta number + label of PC2 location. Also, an equilibrium conformation (*EQ*) was used for comparison, being selected from the unbiased trajectory, where the conformation with dihedral 1 and 2 values closer to the average, was selected. Table 1 reports on the energy differences between states, considering the representative conformations undergoing ESIPT reaction, shown in Figure 2.

From the obtained results, it was possible to observe that the enol emission occurs in the UVA and visible violet regions (325.42 to 391.12 nm). Now, for the keto emissions, it was verified near blue/green emissions (462.63 to 543.73 nm). Therefore, it was observed a large stokes shift between the enol/keto emissions, which is also desirable for promising diagnoses purposes. In this sense, from the spectroscopic and statistical analysis, it is expected to have a higher UVA and visible violet emission for the system with the protein-docked HABT.

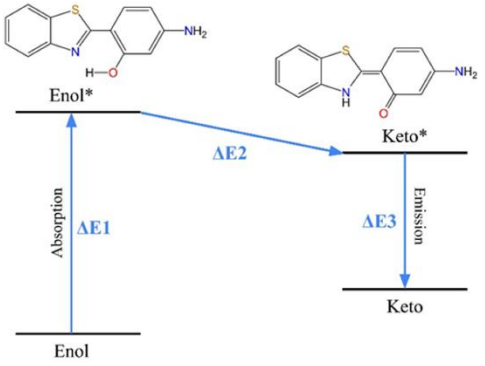

Figure 2. ESIPT scheme, where ΔE1, ΔE2, and ΔE3 represent the energy variation between the states, values which are presented in Table 1.

Table 1. Energy differences between states, in eV, according to the shown in Figure 2.

Conformations	ΔE1	ΔE2	ΔE3
1A	3.98	-	-
1B	3.84	-	-
2A	3.17	-0.03	2.28
2B	3.81	-0.50	2.68
3A	3.56	-	-
3B	3.70	-	-
EQ	*3.37*	*-0.43*	*2.43*

Conclusions

The study demonstrates that HABT exhibits promising ESIPT-based fluorescence behavior, with enol emission increase in the protein environment, suitable for promising cancer diagnosis. In addition, the study shows that from an entirely theoretical methodology, important mechanistic insights could be obtained about PI3K signaling.

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