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# Binding modes in ligand-docked hepatitis B virus core protein simulated by a Monte Carlo method

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**Abstract.** Hepatitis B virus core protein (HBV Cp) is closely involved in the viral assembly, nuclear functions, compartment for reverse transcription, and intra-cellular trafficking. Therefore, modulation of HBV Cp assembly is a promising method to control HBV infection in both preclinical and clinical studies. In this paper, two ligands of HAP18 and AT-130, as representations of heteroaryldihydropyrimidines and phenylpropenamides, have been chosen. Their Binding modes and conformations docking with the HBV Cp have been simulated by a Monte Carlo method. Strong polar contact in HAP18-bound and relatively weak interaction dominated by the Lennard-Jones potential in AT130-bound have been observed, respectively. Although different binding modes result in different assembly behaviors of HBV core protein, both of them strongly influence the quaternary structure of the Cp assembly, changing the spatial relationship between dimers, and inducing noninfective Cp misassembly. The simulation is expected to be helpful to get some insight into the antiviral mechanism of HBV Cp assembly modulation.

**Keywords:** hepatitis B virus core protein, HAP18, AT-130, capsid assembly modulation, binding modes.

## 1. Introduction

According to the World Health Organization, approximate 260 million individuals are infected by hepatitis B virus (HBV) worldwidely and 0.9 million die from HBV-related liver cancer or cirrhosis every year [1]. Drugs, such as the direct-acting nucleotide analogues interfering the immunomodulating interferon alpha and the viral reverse transcriptase, have been used to control the progression of the disease, but they are being hindered in clinics due to their noneffective curative and severe side effects [2].

The DNA of HBV is protected by an icosahedral capsid self-assembled by core protein (Cp) dimers. The capsid plays important part in the viral assembly, regulation and completion of reverse transcription, intra-cellular traffificking, and nuclear functions [3]. Antiviral drugs targeting the Cp have potentials to inhibit assembly of viral particles, viral DNA replication and cccDNA synthesis. Binding small-molecule ligands (core protein allosteric modulators (CpAMs)) to the heteroaryldihydropyrimidine pockets of the HBV capsid can induce Cp dimer misassembling to increase the capsid assembly rate, destabilize the HBV core protein, and result in either aberrant or empty, nonfunctional capsid particles [4,5]. It consequently blocks viral pgRNA and pol packaging into the nucleocapsid and subsequent viral DNA replication. In recent years, as a promising treatment,

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modulation of virus assembly has been attracted much attention, and a variety of novel capsid assembly modulators have been reported [6].

## 2. Methodology

The binding of ligands to large protein receptors is central to numerous biochemical processes. Computer-aided simulation is a powerful tool to predict the binding modes with an acceptable accuracy in the drug design. It is well established that the protein docked by ligand must indicate a structure with the globe minimum free energy. In order to calculate such state, basically, two types of algorithms are applied [7]. One is Molecular Dynamics (MD), which involves in solving the Newton's equations of motions. However, all degrees of freedom need to be considered in MD. If the system contains two sets of degrees of freedom whose characteristic time is far apart, the method will be in dilemma. Furthermore, the calculation results extremely depend on the initial conformation of the system and usually obtain a local minimum energy state, because the molecule trajectory is easily trapped on the rugged hypersurface of the protein. Another one is Stochastic Dynamics (SD), which involves the calculation of the total energy at each possible docking position. Thus, the simulation results of SD no longer depend on the initial conformation of the ligand-receptor system because that a simple energy function is used and the energy barriers on the hypersurface are simply stepped over. Comparing to MD, SD is much efficient because the amount of computation can be reduced attributed to the fact that some degrees of freedom can be replaced by a random force. In this paper, we have simulated a homodimer of HBV Cp docked by two CpAMs with SD technique combining a Monte Carlo search method, which applies random moves of the ligand molecule in the gridded calculation area and accepts/rejects the move based on a Metropolis criterion. The conformation of the docked ligand has been analyzed.

## 3. Results and discussion

The structure of Hepatitis B virus core protein (PDB ID: 1QGT) as the receptor of this simulation was provided by the Protein Data Bank as reported by Leslie et al. [8]. As shown in Figure 1, the HBV capsid with a icosahedral symmetry (T=4) is composed of 60 core protein homodimers. Four Cp monomers which differ slightly from each other in quasiequivalent environments form one basic functional unit of HBV Cp. (Figure 1(b)). Schrödinger 2021-2 was used to carry out the virtual screening. During the preprocessing, missing hydrogens were added to the structure, and the structure was then energy-minimized using OPLS 4. Two small molecule ligands (HAP18 and AT-130), representing heteroaryldihydropyrimidines and phenylpropenamides, respectively, were chosen as CpAMs. Sdf files of the ligands were imported to the workspace. Using LigPrep function in Schrödinger 2021-2, the 3D conformations of two ligands in human plasma of PH=7.4 was simulated with OPLS4 (as shown in Figure 2). It is found that the bonds rotate angles to meet the requirement of the minimum energy state. The Cp receptor was considered as a rigid entity, while the ligands were flexible (the TORSDOF freedom of HAP18 is 7 and that of AT-130 is 6). A cubic grid box with side length of 47.25 angstrom was constructed, as big as possible to cover the whole dimers. A random move, including the move of the ligand mass center along the protein hypersurface in the grid box and the rotation of the bonds, is applied to the ligand. Subsequently, the energy comprising five contributions: Lennard-Jones potential; hydrogen bond; coulombic electrostatic potential; term related to the number of sp3 bonds; and a desolvation term. Finally, the minimized structures are accepted based on the Metropolis acceptance criterion. In the calculation process, step of the random move depends on the curvature of the protein hypersurface assessed by the second derivative of the energy function. Large steps are attempted in areas of small curvature and small steps are attempted in those of large curvature. This Monte Carlo search technique is highly efficient.

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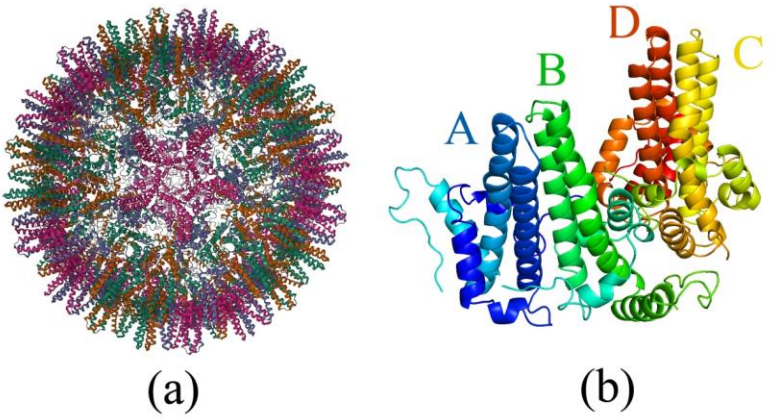


**Figure 1.** Structure of HBV capsid. PDB ID: 1QGT obtained from the Protein Data Bank as reported by Leslie et al. [8]. (a) The quaternary structure of an HBV capsid. (b) Close-up of a homodimer composed of AB dimer and CD dimer, or chain A-blue, chain B-green, chain C-yellow and chain D-brown.

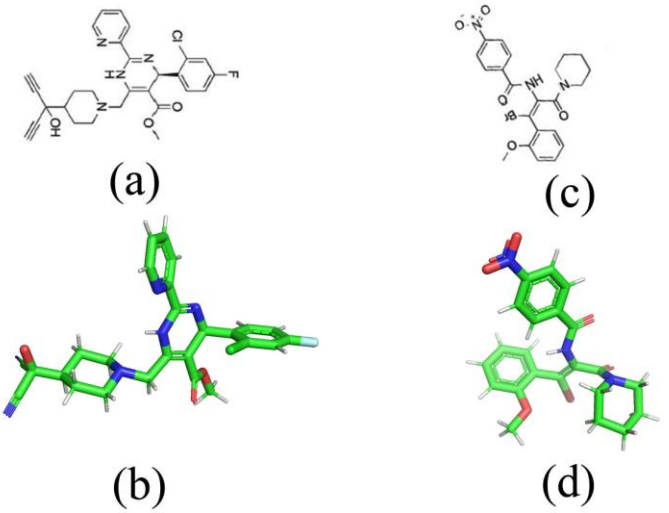


**Figure 2.** Ligands of HAP18 (a) and AT-130 (c) and their 3D conformations in human plasma of PH=7.4 simulated with OPLS4, (b) HAP18, (d) AT-130, the different atoms are colored: carbon-green, oxygen-red, nitrogen-blue, and hydrogen-gray.

Figure 3 indicates that there are two binding sites on the Cp homodimer for the HAP18 docking. One is located between chain A and chain B (Figure 3(a)), while another one binds to the hydrophobic pocket on the dimer-dimer interface (Figure 3(b)). Two polar contacts between oxygen and nitrogen atoms are observed, inferring a strong ligand-protein interaction. Such binding enhances the stability of the intra-protein interactions, increases the rate of HBV core protein assembly, and induces the formation of large, regular complexes as reported by Bourne C. et al. [9]. Figure 4 shows the AT130-bound core protein. The binding sites are located the dimer-dimer interfaces (between chain A and chain D for docked AB dimer (Figure 4(a)) and between chain B and chain C for docked CD dimer (Figure 4(b))). The AT130-protein interaction is much weaker than that of HAP18-bound, no polar contact is discovered. The Lennard-Jones interaction results in bonds rotation so that AT130 molecule form a conformation fitting the rugged protein hypersurface. It has been found that both HAP18 and AT-130 binding to HAP pockets can lead to quaternary structural changes in the nucleocapsid, but HAP18 induces a much more significant quaternary rearrangement than AT-130 due to the stronger interaction

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between HAP18 and receptor. AT-130 can also compensatively cause tertiary structural changes during nucleocapsid assembling [10]. Such effects should be related to the binding mode induced by two ligands.

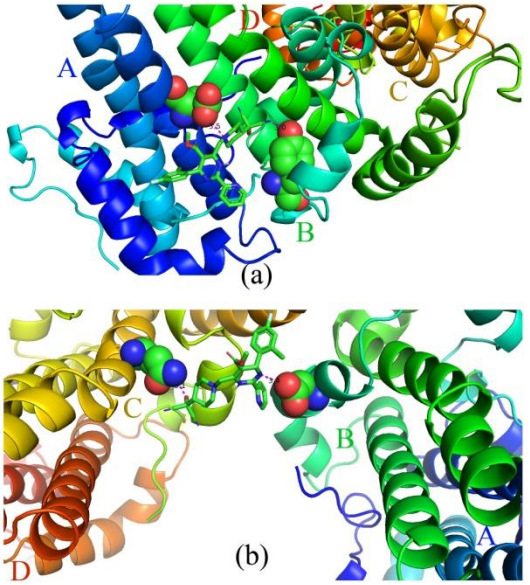


**Figure 3.** Simulated optimal binding modes and conformations of (a) HAP18 with AB dimer and (b) with CD dimer, respectively. Oxygen-Nitrogen polar contacts are indicated with red dash lines. Biding sites appear at chain-chain interface (a) and dimer-dimer interface (b).

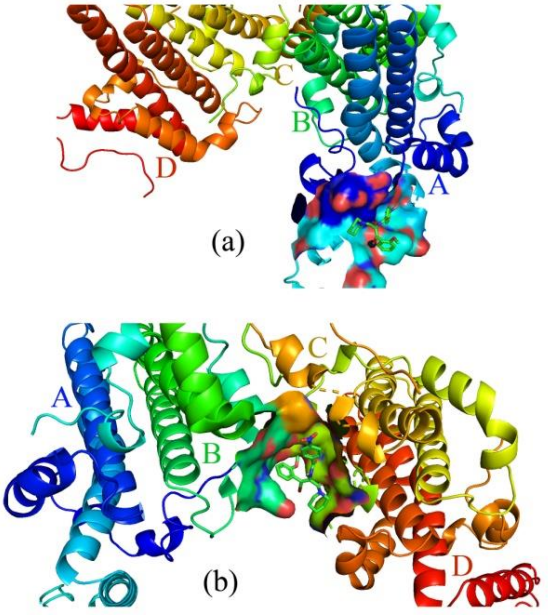


**Figure 4.** Simulated optimal binding modes and conformations of (a) AT-130 with AB dimer and (b) with CD dimer, respectively. The binding sites are located the hydrophobic pockets on the dimer-dimer interfaces: (a) between chain A and chain D for docked AB dimer and (b) between chain B and chain C for docked CD dimer.

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## 4. Conclusion

In conclusion, chosen ligands of HAP18 and AT-130 were chosen to represent two different types of CpAMs and to dock with the HBV capsid homodimer. The binding modes and conformations of them have been simulated with a Monte Carlo method, which is stochastic and ergodic but computational time saving. It has been found that HAP18 prefers to a polar contact with the HBV capsid, binding to either the interchain site at the AB dimer or the hydrophobic pocket on the AB-CD dimer interface. AT-130 prefers to a relatively weak Lennard-Jones interaction, binding to the hydrophobic pockets at the dimer-dimer interface. The difference in the interactions between ligands and receptor has resulted in different effects on the conformation of dimer assembly. Both of them lead to quaternary structural changes in the nucleocapsid, and then altering the spatial relationship between dimmers, but HAP18 induces a much more significant quaternary rearrangement than AT-130 due to the stronger interaction. AT-130 can compensatively cause tertiary structural changes during nucleocapsid assembling. These changes are large enough to cause noninfective Cp misassembly. The simulation results have indicated that the bindings of HAP18 and AT-130 are not competitive at Cp hypersureface. This paper therefore proposes that multiple ligand-binding is worth trying to improve the effects of CpAMs.

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