MOLECULAR DYNAMICS SIMULATIONS OF THE HBV CAPSID

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EXECUTIVE SUMMARY

The hepatitis B virus (HBV) is a major cause of liver disease. The World Health Organization estimates that more than 250 million persons currently suffer from chronic infection, with no cure available. Researchers aim to develop new treatments targeting HBV's capsid, the protein shell that encloses its viral genome (Fig. 1). The research team has been leveraging Blue Waters since 2015 as a computational microscope to study the capsid, employing all-atom molecular dynamics simulations to reveal details of structure and function that are inaccessible to experiments.

Currently, the team is investigating the capsid's response to mutations and drug compounds, probing its mechanics to discover capabilities and potential vulnerabilities. The researchers have learned that amino acids aspartate-78 and threonine-109 each play important, heretofore, unrealized roles in capsid assembly, and the team has implicated threonine-109 in drug resistance. Heteroaryldihydropyrimidine (HAP) compounds disrupt the capsid's shape, volume, and solvent transport properties. Importantly, simulation of the multimillion-atom capsid system is only possible on a petascale supercomputer such as Blue Waters.

RESEARCH CHALLENGE

The HBV capsid is a complex molecular machine. It self-assembles from 120 capsid-protein dimers to package RNA, facilitates the maturation of the RNA to DNA, and hijacks various components of the host cell's own machinery to transport its genomic cargo throughout the viral infection cycle. Drugs that disrupt the capsid have been identified but have not been approved for human use. The ability to produce new treatments that target the capsid depends heavily on understanding its inner workings and the mechanisms by which it carries out its function; by determining how the capsid works, researchers can also determine how best to inhibit it.

METHODS & CODES

Molecular dynamics simulations provide a powerful tool to investigate virus capsids such as that of HBV [1]. This project has demonstrated that when performed at all-atom resolution, simulations can capture remarkably subtle details of capsid structure and dynamics, including changes induced by bound drugs [2]. The simulations employed NAMD [3], a highly scalable biomolecular simulation code with a long and successful track record of deployment on Blue Waters. While all-atom simulations of the intact HBV capsid come at a great computational expense, access to NAMD on Blue Waters has enabled the research team to reveal critical new insights into its function and suggest strategies for targeting it with novel therapeutics [4,5].

RESULTS & IMPACT

Aspartate-78. The surface of the HBV capsid exhibits 120 spikes (Fig. 1), one for every incorporated capsid-protein dimer. The tips of the spikes contain four negatively charged amino acids: two copies each of glutamate-77 and aspartate-78 (Fig. 2a). The research team's collaborators hypothesized that mutation of aspartate-78 to an uncharged amino acid would promote capsid assembly by decreasing electrostatic repulsion; however, experiments indicated that substitution of aspartate with serine was detrimental to the formation of capsids.

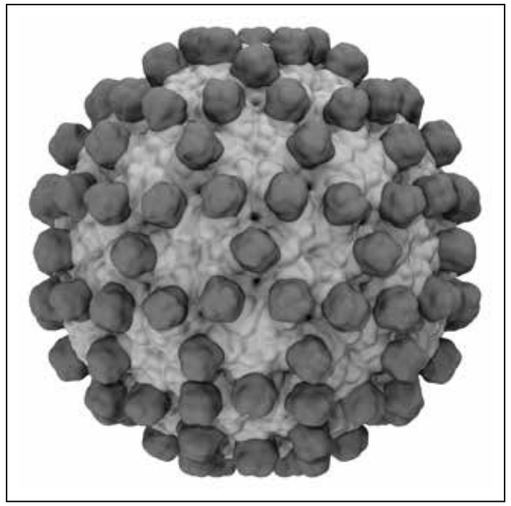

Figure 1: The HBV capsid is composed of 120 capsid-protein dimers. Each dimer contains a spike domain, shown here in dark gray.

The research team used Blue Waters to simulate multiple copies of wild type and mutant HBV capsid-protein dimers, obtaining conformational sampling totaling six microseconds. The team's analyses showed that together, glutamate-77 and aspartate-78 induce sodium localization within their vicinity and transiently coordinate individual sodium ions within the spike tip (Fig. 2a). Substitution of aspartate-78 with serine caused the spike interface to open, dramatically reducing sodium localization and coordination. Both simulations and cryo-electron microscopy data showed that the mutation increased disorder in the spikes, demonstrating that aspartate-78 and its ion-mediated interactions are important for maintaining spike secondary structure and conformation conducive to productive capsid assembly.

Threonine-109. A number of drugs that target the HBV capsid are known to interfere with its assembly process. Experiments by the research team's collaborators indicated that mutation of the amino acid threonine-109 (Fig. 2b) could increase both the rate of capsid assembly and the capsid's resistance to assembly-disrupting drugs; however, no explanation for threonine-109's role in either aspect was apparent from high-resolution crystal or cryo-electron microscopy structures of the capsid.

The research team used Blue Waters to simulate the intact HBV capsid on the microsecond timescale [4], producing an ensemble of 12 million samples that characterized the dynamical behavior of threonine-109. The team's analyses showed that threonine-109 spends a significant amount of time mediating contact among neighboring dimers within the capsid (Fig. 2b), revealing why mutation of this amino acid can alter the ability of the proteins to interact and assemble. Further, the team found that the interdimer contact formed by threonine-109 can occlude a hydrophobic pocket recognized by capsid-disrupting drugs (Fig. 2b). Threonine-109's ability to physically block the binding of such compounds confers some native drug resistance to HBV. Mutation of threonine-109 to larger, more hydrophobic amino acids enhances the rate of capsid assembly and also increases drug resistance.

HAP compounds. Drugs from the heteroaryldihydropyrimidine (HAP) family (Fig. 2b) can misdirect HBV capsid assembly and disrupt intact capsids. The research team previously used Blue Waters to simulate a HAP-bound capsid and observed that the compounds induced changes in the capsid's shape [2] consistent with experimental observations. Now, the team has extended the drug-bound capsid investigation to the microsecond timescale, treating HAP as a small-molecule probe to test the capsid's mechanics. The results indicate that saturation with HAP compounds increases the capsid's volume and alters its solvent transport properties, revealing insight into the mechanism by which HAPs induce disruption. The team's analyses were enabled by a novel method on which the researchers collaborated that uses ray-casting to accurately detect the interior versus exterior space of biomolecular containers, including virus capsids. The method is highly parallelizable and takes advantage of Blue Waters' Lustre filesystem.

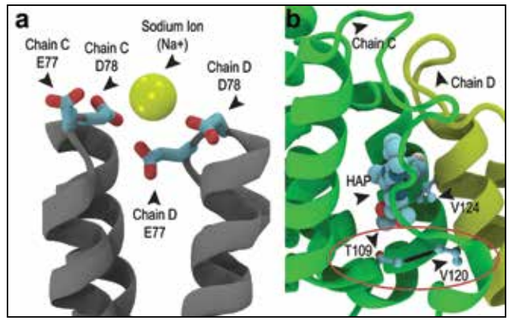

Figure 2: (a) The HBV capsid's spikes contain glutamate-77 and aspartate-78. The team discovered that these negatively charged amino acids can transiently coordinate positively charged ions such as sodium. (b) The team also discovered that threonine-109 can form interdimer interactions that can block the binding of capsid-disrupting compounds such as HAPs to confer drug resistance.

WHY BLUE WATERS

Simulations of the intact HBV capsid are only feasible on a petascale supercomputer such as Blue Waters because of their computational expense. Investigating the capsid under physiological conditions at full chemical resolution requires calculations involving the interactions of millions of atoms. Simulations exploring the microsecond timescale can take months, even on thousands of processors. The exciting discoveries revealed by this work underscore the essential role for leadership-class computing resources in supporting basic science research toward gaining an understanding of viruses and developing novel antiviral treatments.

PUBLICATIONS & DATA SETS

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