VIRTUAL FLU, A DIFFERENT KIND OF COMPUTER VIRUS

FIGURE 1: The influenza surface coat. The hemagglutinin and neuraminidase glycoproteins are represented in lavender and magenta, respectively. The enveloping lipid bilayer is represented in glossy pink. Credit: Jacob D. Durrant.

Allocation: NSF PRAC/6.00 Mnh
PI: Rommie E. Amaro[1]
Collaborator: Alasdair Steven[2]

[1]University of California, San Diego
[2]University of Illinois at Urbana-Champaign

EXECUTIVE SUMMARY

Influenza infection is routinely responsible for hundreds of thousands of deaths annually, punctuated by catastrophic pandemics roughly every 25 years. Some experimental techniques used to study the virus suffer from serious drawbacks. For example, structural-biology methods like electron microscopy, X-ray crystallography, and nuclear magnetic resonance spectroscopy (NMR) typically cannot provide either the atomic resolution, size scaling, or molecular-dynamics data required to answer a number of pharmacologically important questions.

To address these concerns, we have constructed an atomic-resolution model of the **entire influenza viral coat**, containing 160 million atoms. We recently simulated this large-scale system for 121 nanoseconds in order to gain a more complete understanding of the influenza infection process. This research now allows us to explore novel opportunities for drug and vaccine development *in silico*.

INTRODUCTION

We focused our research on the viral surface coat because of the important role it plays in both the initial and final stages of the influenza infection process. The viral coat is composed of a lipid bilayer from which two spike-like glycoproteins, neuraminidase and hemagglutinin, protrude (Fig. 1). When a viral particle first approaches a human host cell, the hemagglutinin proteins latch onto sialic acid molecules attached to the cell surface, ultimately leading to the molecules being transported into the cell. A second surface-coat protein, the M2 proton channel, acidifies the virus interior once the virion enters the endosome pocket within the cell, triggering conformational changes that facilitate replication of the virus [1]. Following replication, the viral progeny bud from the host cell but remain attached to its external surface by the same sialic acid connections. Influenza's neuraminidase glycoprotein is responsible for severing those tethers, allowing the newly formed viruses to depart and infect the next cell [2].

The viral coat has been extensively studied precisely because it is so critical for infection. By analyzing the molecular structures of the various components of the surface coat in isolation, researchers have produced a number of anti-flu drugs currently used clinically (e.g., Tamiflu). Unfortunately, the flu is highly adaptable, and resistance to these medicines has already been documented [1, 3-5]. There is an urgent need for novel therapeutics. Studying the structures and motions of the various surface-coat components when assembled into their natural multi-component environment, rather than in isolation, provides pharmacologically relevant insights that will help us combat future pandemics. No current experimental technique is capable of providing an accurate spatial and temporal model of the entire *dynamic* surface coat at the resolution needed for drug discovery; fortunately, modeling and simulation can serve as a "computational microscope" that provide the needed information.

METHODS & RESULTS

Guided by experimental data, we constructed a surface-coat model of the 2009 H1N1 pandemic flu strain. When immersed in a bath of virtual water with the appropriate electrolytes, each system contains over 160 million atoms (Fig. 2). Our collaborator Alasdair Steven at the University of Illinois at Urbana-Champaign used electron microscopy to identify the general shape of the influenza virus and the approximate locations of the glycoprotein spikes [6]. We then used computational methods developed in our lab to wrap this virus volume in a virtual lipid bilayer [7] and to position atomic-resolution models of the glycoproteins at the appropriate locations. An appropriate number of M2 channels were also added to the structure. We were thus able to transform the low-resolution microscopic data into a high-resolution, atomistic model suitable for molecular dynamics simulations and, ultimately, drug discovery. We finished simulations of this virtual virus several months ago on Blue Waters. Ongoing and preliminary analyses of the simulations have suggested several interesting findings. First, we analyzed the apo neuraminidase molecules scattered across the virion surface. The R371 and R292 residues, which form electrostatic interactions with most endogenous and pharmaceutical ligands, sample many apo conformations other than that of the crystal structure, suggesting that binding occurs via a conformational-shift mechanism. The

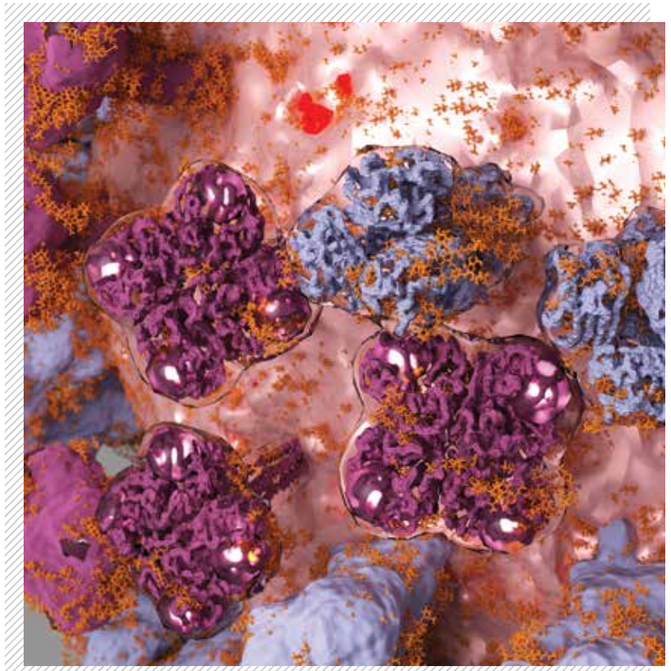

FIGURE 2: The viral surface coat. Selected neuraminidase and hemagglutinin glycoproteins are rendered transparent, revealing the underlying protein backbone. An M2 proton channel, in red, is also visible. This image highlights the fact that these renderings were generated from high-resolution (ultimately atomic-resolution) data. Credit: Jacob D. Durrant.

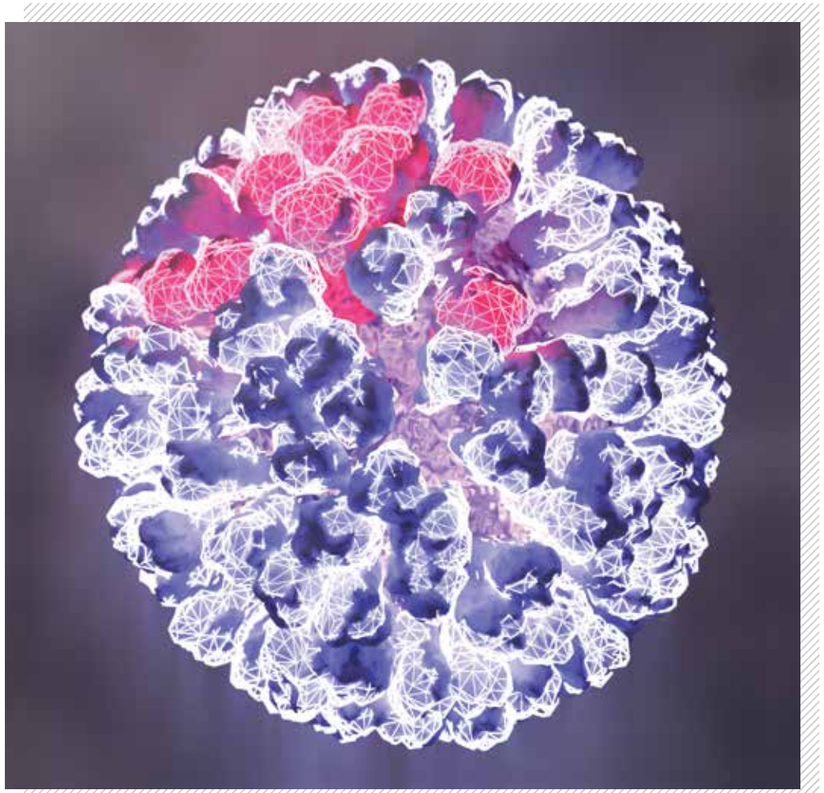

FIGURE 3: Surface-coat dynamics: the first and last frames of the whole-virion simulation. Neuraminidase and hemagglutinin are shown in red and blue, respectively. The glycoprotein conformations of the first and last frames are shown in solid and glowing-mesh representations, respectively. As expected for a simulation of this duration, glycoprotein diffusion through the lipid bilayer was limited. However, the atomic-resolution motions of individual glycoproteins were substantial because they were sampled over both simulated time and space, thanks to the multiple copies of each glycoprotein scattered across the model surface. Credit: Jacob D. Durrant.

motions of these residues permit conformations that have additional druggable hotspots beyond those of the sialic acid-binding and 150-loop regions. Furthermore, we are currently building Markov state models to explore the pharmacologically relevant kinetics of 150-loop opening and closing, and the neuraminidase conformations sampled by this large-scale simulation may prove useful for future virtual-screening efforts as well.

We are similarly analyzing the many hemagglutinin molecules included in the whole-virion-coat simulations. We are hopeful that Brownian dynamics simulations in the context of the whole viral particle will provide useful insights into the mechanism of broadly neutralizing antibodies.

The M2 channels of our model also sample many conformations, ranging from open to closed. We have characterized the volume distributions of these channels and hope to build a Markov state model to describe the opening/closing kinetics of this crucial surface-coat component. The many M2-channel conformations sampled may also prove useful in future small-molecule virtual screens.

WHY BLUE WATERS

Blue Waters has been critical for this project. To our knowledge, a molecular dynamics simulation on so grand a scale has **never before** been attempted. Very few supercomputers are capable of the petascale performance required. **Without Blue Waters, the current work would be impossible.**

These simulations are providing important information about the surface motions and electric fields that surround the viral particle. These "dynamics" and "electrostatics" govern not only the infection process, but also drug and vaccine/antibody binding.

MOLECULAR DYNAMICS OF SELF-ASSEMBLED DNA SYSTEMS

Allocation: Blue Waters Professor/240 Knh
PI: Aleksei Aksimentiev[1]
Collaborators: Chen-Yu Li[1], Jejoong Yoo[1], Christopher Maffeo[1], Scott Michael Slone[1]

[1]University of Illinois at Urbana-Champaign

EXECUTIVE SUMMARY

DNA nanotechnology utilizes self-assembly for the high-throughput construction of sub-micron-size objects with nanometer precision. In comparison to conventional nanofabrication approaches, the DNA origami method is relatively low cost, easy to use, and has an infinite number of possible applications. Using Blue Waters, we have explored the ability of DNA nanostructures to function as membrane channels, carried out a **landmark simulation** of a DNA origami sculpture, and characterized the mechanical properties and ionic conductivity of DNA brick structures. The results of our simulations have contributed to the development of a web server for prediction of DNA origami structures and a web tool for designing nanostructures using the DNA brick methods.

INTRODUCTION

DNA origami is an experimental technique that allows folding of a long DNA molecule into an arbitrary three-dimensional shape [1]. Over the past ten years, the DNA origami method has **advanced** to encompass self-assembly of complex 3D objects with sub-nanometer precision including static structures [2], as well as objects that perform active functions [3].

Predictive computational modeling of DNA origami is an attractive alternative to experimental characterization of such self-assembled objects. Currently, the most accurate computational method is all-atom molecular dynamics (MD). In 2013, we reported the **first** MD study of several model DNA origami systems [4]. Last year, we explored the possibility of using DNA origami in nanopore sensing applications [5]. Our most recent work includes a study of a DNA-based channel embedded in a lipid bilayer, a **landmark** simulation of a DNA sculpture that demonstrates the predictive power of the MD method, and a detailed comparison between DNA origami and an alternative self-assembly approach known as DNA bricks.

METHODS & RESULTS

In living cells, membrane protein channels control the transport of molecules across the cell membrane. Recently, several experimental groups demonstrated assembly and insertion of DNA channels into lipid bilayer membranes [6]. A typical DNA channel is made by arranging several parallel DNA double helices to form a polygon. The central cavity of the polygon is the transmembrane pore. Using

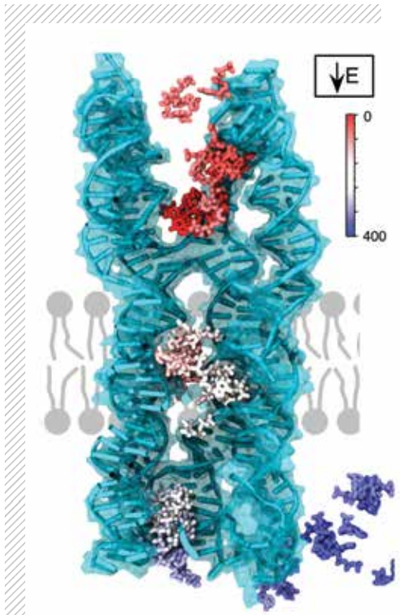

FIGURE 1: A time-lapse illustration of the MD trajectory showing an ATP molecule passing from one side of the DNA channel to the other. The color indicates the progress of the simulation using the red (beginning)–white–blue (end) scheme. Because of the electro-osmotic flow, the ATP molecule moves in the direction of the electric field, opposite to the direction prescribed by its negative charge.