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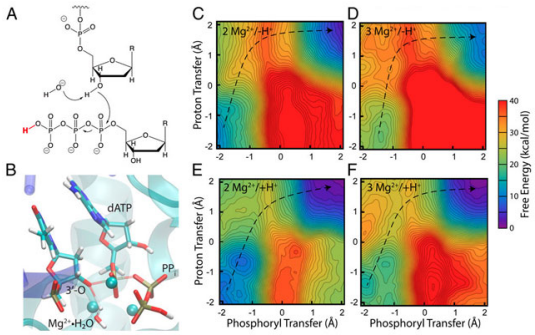
Supercomputers Unlock Reproductive Mysteries of Viruses and Life

Simulations pertinent to Coronavirus, DNA replication

Viruses such as the dreaded severe acute respiratory syndrome coronavirus 2 rely on the host cell membrane to drastically bend and eventually let loose the replicated viruses trapped inside the cell. Scientists recently used supercomputer simulations to help propose a mechanism for this budding off of viruses.



Mechanism (A), transition state structure (B), and free-energy surfaces for a mechanism with a Mg2+-coordinated hydroxide as the base under 4 different conditions (C–F). The 4 conditions are: 2 Mg2+ and deprotonated leaving group (C), 3 Mg2+ and deprotonated leaving group (D), 2 Mg2+ and protonated leaving group (E), 3 Mg2+ and protonated leaving group (F). The proposed mechanism achieves all the characteristics of the mechanism suggested by experiments, including rate acceleration by a third Mg2+ and by protonation of the leaving group. The structure in the Upper Right is representative of the transition state region for that reaction with the breaking and forming bonds shown as transparent. The proton shown in red is only present in the Bottom simulations. The dotted lines guide the eye along the minimum free-energy path from reactant to product; the transition state corresponds to the location of the maximum free energy along this minimum path. Credit: Roston et al.

Moreover, a related study also relied on supercomputer simulations to find a mechanism for how the DNA of all life adds a base to its growing strand during replication. This fundamental research could help lead to new strategies and better technology that combats infectious and genetic diseases.

The study on cell membrane remodeling, important for viral reproduction, cell growth and communication, and other biological processes was published online in February 2020 in the *Biophysical Journal*. Qiang Cui, a professor in the Departments of Chemistry, Physics, and Biomedical Engineering at Boston University and the study's co-author, also was part of a study on DNA base addition, published in the December 2019 *Proceedings of the National Academy of Sciences*.

Cui's science team developed supercomputer simulations of the cell membrane, in particular filaments of the Vps32 protein, a major component of the endosomal sorting required for transport complex (ESCRT-III), which was the prime suspect for the driving force that causes the cell membrane to form buds in a process called membrane invagination. ESCRT proteins function in the cytosol, the liquid inside cells surrounding organelles, the cell subunits. They perform various jobs such as making organelles; sorting recyclable material in the cell and ejecting waste, and more.

"Supercomputers with massive parallelization are very much required to push the boundary of bimolecular simulations," said Cui, who was the principal investigator on both studies.

Electron microscopy shows the Vps32 protein polymerizes, or assembles itself into a corkscrew shape during membrane invagination. The study authors sought to establish whether the atomistic forces inside Vps32 cause it to bend and twist, ultimately tugging and budding off the membrane. Unfortunately, experimental studies currently lack the resolution to characterize the protein-membrane interactions that lead to the membrane deformations.

The research team used atomistic molecular dynamics simulations to investigate protein-protein interfaces in one-dimensional filament structures in solution and to find the residues holding the filament together. They also studied the protein-membrane interface using a Vps32 trimer model.

"I think the most interesting observation is that the ESCRTIII polymer that we studied features a clear intrinsic twist," said Cui. "This suggests that twisting stress that accumulates as the polymer grows on the surface might play a major role in creating the three-dimensional buckling of the membrane. People focused more on the bending of the filament in the past."

Simulations of systems containing up to two million atoms posed a large hurdle for Cui and colleagues. Researchers completed their simulations on the *Stampede2* system at the Texas Advanced Computing Center of UT Austin. "*Stampede2* has been crucial for us to set up these relatively large-scale membrane simulations," said Cui.

While this study is pure research, the knowledge gained could help benefit society. "Membrane remodeling is an important process that underlies many crucial cellular functions and events - such as synaptic transmission and virus infection," explained Cui. "Understanding the mechanism of membrane remodeling will ultimately help propose new strategies for battling human diseases due to impaired membrane fusion activities – or preventing viral infection – a timely topic these days given the quick spread of the new coronavirus."

DNA Determinations

Cui also co-authored a computational study that used supercomputer simulations to determine a chemical mechanism for the reaction of nucleotide addition, used in the cell to add nucleotide bases to a growing strand of DNA. “By doing that, computationally, we are also able to determine the role of a catalytic metal ion of magnesium that’s in the active site of the enzyme DNA polymerase,” said study co-author Daniel Roston, an assistant project scientist in the Department of Chemistry and Biology at UC San Diego. “This metal has been a bit controversial in the literature. Nobody was really sure exactly what it was doing there. We think it’s playing an important catalytic role.”

DNA polymerase adds the nucleotides guanine, adenine, thymine, cytosine (G-A-T-C) to DNA by removing a proton from the end of the growing strand through reaction with a water molecule. “When we say in the study that a water molecule serves as the base, it serves as a base to remove a proton, an acid base chemistry. What’s left there after you remove the proton is much more chemically active to react with a new nucleotide that needs to be added to the DNA,” said Roston.

The chemistry needs multiple proton transfers in a complex active site. Experimental probes using X-ray crystallography have been unable to distinguish among the many possible reaction pathways.

“Simulations offer a compliment to crystallography because you can model in all the hydrogens and run molecular dynamics simulations, where you allow all the atoms to move around in the simulation and see where they want to go, and what interactions are helping them get where they need to go,” explained Roston. “Our role was to do these molecular dynamics simulations and test different models for how the atoms are moving around during the reaction and test different interactions that are helping that along.”

“Chemical reactions, life, doesn’t happen that quickly,” said Roston. “It happens on a timescale of people talking to each other. Bridging this gap in timescale of many, many orders of magnitude requires many steps in your simulations. It very quickly becomes computationally intractable.”

Roston and colleagues used about 500,000 CPU hours on the *Comet* supercomputer at the San Diego Supercomputer Center (SDSC) on the UC San Diego campus, with the allocation provided via the National Science Foundation’s XSEDE (Extreme Science and Engineering

Discovery Environment) program. *Comet* enabled them to simultaneously run many different simulations that all feed off one another. “I’m not sure who else would have given me that much computer time,” Roston said. “We were lucky to be able to have this resource for the project.”

“DNA replication is what life is about,” said Roston. “We’re getting at the heart of how that happens, the really fundamental process to life as we know it on Earth. This is so important, we should really understand how it works at a deep level. But then, there are also important aspects of technology such as CRISPR that take advantage of this kind of work to develop systems to manipulate DNA. Understanding the details of how life has evolved to manipulate DNA will surely play a role in feeding our understanding and our ability to harness technologies in the future.”

The ESCRT-III study co-authors are Taraknath Mandal and Qiang Cui of Boston University; Wilson Lough, Saverio E. Spagnolie, and Anjon Audhya of the University of Wisconsin - Madison. Funding came from the National Science Foundation. Computations are also supported in part by the Shared Computing Cluster, which is administered by Boston University’s Research Computing Services.

The DNA study co-authors also include Darren Demapan of the University of Wisconsin - Madison. Funding was provided by the National Institutes of Health.

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