Studies with Anton, a special-purpose supercomputer designed by D. E. Shaw Research and made available to the research community through PSC, have yielded invaluable insights into the motion and function of proteins.
Enter Anton: a supercomputer designed and built – by D. E. Shaw Research (DESRES) in New York City – to dramatically increase the speed of MD. Named in honor of Dutch microscope inventor Anton van Leeuwenhoek, Anton allows researchers to see previously unseen biomolecular activity. By executing ultrafast MD, Anton simulates proteins (and nucleic acids) for longer stretches of biological time than was previously possible. Before Anton, even the most powerful supercomputers could – because of the prohibitive amounts of computing required – only hundreds of nanoseconds (10⁻⁹ seconds), with a few MD simulations reaching into the microsecond range (10⁻⁶ seconds).

“Anton performs MD simulations up to 100 times faster than conventional supercomputers,” says Markus Dittmer of PSC’s National Resource for Biomolecular Supercomputing (NRBSC), who directs the Anton program at PSC, “making it possible for the first time to simulate the behavior of proteins over more than a millisecond of biological time. The availability of these extended timescales has opened a new window on many important biological processes.”

Thanks to DESRES, which provided a machine to NRBSC without cost, an Anton system has been available at PSC since late 2010 for use by the general biomedical community. A two-year $2.7 million grant from NIH’s National Institute of General Medical Sciences provided initial support for operational costs. To date, 70 research groups have used Anton at PSC for work on 91 projects, with a new round of projects described in the rest of this article, has led to many new findings.

The Protein-Folding Problem: A Big Step

A droopy, strung-out chain of amino acids – that’s what rolls off the assembly line of the molecular factory inside a cell when a protein is created. All the pieces are there, and they’re in the right sequence. But the new protein is unfit for duty.

To do its job, this dangly chain must fold into just the right three-dimensional configuration. It happens within seconds or less, and the result is a complex bundle of helices, sheets and turns ready-made for the protein to go to work.

How does this happen? Out of billions of possible shapes that the chain of amino acids could assume, how does it arrive at the shape it takes in nature? “It’s a famous problem,” says biophysicist Klaus Schulten of the University of Illinois, Urbana-Champaign, “and only recently, because of computing, has it been coming close to solution. Experiments measure too few specifics of this process to make a statement. Only the computer, with MD simulations, can follow the detailed processes involved when strands of amino acid arrange themselves into a protein in proper form.”

Using Anton, Schulten – teamed with experimentalist Martin Graebeke and UIC colleagues, successfully simulated the folding of an 80 amino-acid protein (lambda-repressor). With water molecules and ions, the simulation included 74,253 atoms. Their findings (Journal of Physical Chemistry Letters, April 2012) showed a folded result in good agreement with experiments, and went beyond experiment to find that accepted ways of measuring folding in the laboratory give an incomplete picture. Further experimental work, and a follow-up paper, are underway.

“This field is undergoing a revolution” says Schulten. “It began with folding very small proteins, but now with Anton, we’re able to fold larger, more natural proteins. Lambda-repressor is one of the largest proteins the folding of which has been monitored and described in the computer. It’s a stepping stone toward solving the very important protein-folding problem.”

The researchers in total tracked 100 microseconds of protein movement, 80 microseconds with Anton – in two separate 40 microsecond simulations, each of which took a week. The additional 20 microseconds with another computer took a year, explains Schulten: “We could do this only with Anton.”

Stop the Bleeding: Inside-Out Signaling

Integrins are the essential two-way communicator proteins of cellular biology. This large family of receptors, proteins that reside in the cellular membrane, receive information about things happening in the cell’s external environment, the extracellular matrix (ECM), which triggers a cellular response. For example, integrins marshal the body’s response to a wound. When exposed to collagen – proteins in connective tissue – at a wound site, integrins on the surface of blood platelet cells change shape, a shift that dramatically increases integrin’s binding affinity for fibrinogen, a blood clot forming protein. Through this process, called thrombosis, fibrinogen binds platelets to each other, a blood clot forms, and – if all goes well – bleeding stops.

Integrins are also involved in cell migration and immune-system patrolling, among other processes, and along with responding to changes in the ECM, integrins also operate in an “inside-out” mode of signaling. They switch to a different shape – become activated – through interaction with intracellular proteins, one of which is called talin.

“Talin binds to the tail of integrin,” says Marta Filizola of Mount Sinai School of Medicine, “and it’s a hard question to determine how and to what degree this has an impact on activating integrin.”

Using Anton, Filizola and colleagues simulated the helical region of the platelet integrin (called αβ3) that lodges in the cellular membrane with a tail extending into the cell. Their MD simulations included a phospholipid bilayer, representing the cell membrane, surrounding water molecules and ions, along with two talin domains (F2 & F3) – an unprecedented level of detail, about 76,000 atoms. They simulated about five microseconds of talin interacting with integrin and, for contrast, did the same simulation of integrin and the lipid membrane without the presence of talin, about 58,000 atoms. The researchers augmented this second simulation with additional MD simulations on their inhouse cluster.

The showed atom-by-atom details of the talin-integrin interaction give new and previous understanding.

Their studies confirm a hypothesis that had been put forward by recent experimental work – that parts of talin anchor to the inner membrane wall, helping to stabilize the interaction with integrin. They also showed atom-by-atom details of the talin-integrin interaction that go beyond previous understanding and suggest directions for more experimental studies. Specifically, they showed, Filizola, that talin reduces a tilted orientation of integrin’s two helical subunits, and induces bending of integrin’s β3 helix. “These simulations, which we couldn’t have done without Anton, broaden our understanding of how talin contributes to integrin’s activation.”
Opening the Gate to Action

When the starting gun sounds and Usain Bolt explodes from the blocks in the 100-meter dash, electrical signals in the brain trigger his blastoff. Voltage-gated ion channels open in nerve cells, and ions—charged atoms, usually sodium and potassium—flow through the opened gates, creating electrical currents that cause muscle fibers to contract.

“Every communication in the central nervous system is possible because ions flow across the cell membrane,” says Alfredo Freites, a biophysicist with the group of Douglas Tobias at the University of California, Irvine. The ions flow, he explains, through what’s essentially a hole in the membrane formed by proteins, called voltage-gated ion channels. These channels open and close based on the ability of part of the protein, called the “voltage-sensing domain” (VSD), to respond to changes in electrical potential.

Laboratory studies over many years have shown that currents called “gating currents”—in the VSDs are associated with motions that trigger opening of the channel. Until the availability of Anton, however, it hadn’t been possible to track what structural changes happen in the VSD during the gating event. “Despite a wide variety of data,” says Freites, “the molecular mechanism of voltage gating hasn’t been well understood.”

With Anton, Freites and his colleagues Eric Schow, Stephen White and Douglas Tobias were able to run MD simulations over a timescale that corresponds to a VSD gating-current event. They simulated the VSD embedded in a lipid bilayer, representing the cellular membrane, along with surrounding water with an applied electric field for a period of 30 microseconds. At this timescale, the researchers were able to make direct comparisons between MD simulations and laboratory data. “With any other high-performance computing resource,” says Freites, “it would be impractical to do this.”

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Their findings—reported in The Biophysical Journal (June 2012)—were, in general, consistent with the data from laboratory studies. The detail of the MD results suggests, nevertheless, that gating-charge measurements from electro-physiological lab studies “may not represent a single-charge displacement but may instead be the superposition of many events occurring faster than the instrument response.” Their findings also go beyond prior studies, observes Freites, in showing that the presence of water molecules within the VSD is necessary for the gating current to flow and pull the channel open. “Water facilitates the flow of charges,” says Freites, “and we see that the VSD’s own hydration is what allows this event to happen seamlessly.”

Motion at PKA’s Active Site

This graphic shows overlaid snapshots from one of the simulations by McClendon and Taylor. The two main structural components of PKA’s catalytic domain, the N-lobe (light gray) and C-lobe (dark green), enclose the “active site,” which holds ATP (black) and two magnesium ions (purple). The image, observes McClendon, shows that PKA’s glycine-rich loop (C) and nearby R-helix (B) “aren’t locked down by the ATP and two magnesiums, but instead remain more flexible than we expected.”

Action at the Crossroads

Think of a traffic cop at a crazy downtown five-way (or more) intersection. The officer in blue is performing a graceful dance—swaying care-free through one direction, holding them off from another, switching from stop to go, to stop, vehicles and their payloads of goods and busy people on intersecting paths getting to where they need to go much more smoothly and expeditiously than you thought possible.

In a cell of the human body, one such traffic cop is the protein kinase A (PKA)—a crude analogy, but one that roughly conveys the role played by this protein in regulating the complex network of chemical reactions within the cell. PKA is one among a superfamily of enzymes, the kinases, that are ubiquitous in living things. “Protein kinases operate like stop and go signals,” says Susan Taylor, professor of chemistry and biochemistry at the University of California, San Diego. “They are essential molecular switches for all biology.”

PKA is the prototype of this big family, a protein for which Taylor and her colleagues first solved the structure in 1991. With this structure as a map, Taylor’s research group has answered many questions about how protein kinases regulate cell metabolism by means of a biochemical handoff called “phosphorylation.” Protein kinases take the cell’s energy-carrier molecule, ATP (adenosine triphosphate), and transfer a phosphate group from it to target proteins, often altering the target proteins’ functions. Having given up a phosphate, the ATP becomes ADP (adenosine diphosphate). Kinases then let go of the ADP and become active again once they bind with another ATP molecule and find another target protein to phosphorylate.

By phosphorylating a variety of target proteins, PKA helps regulate memory, cell growth and many other processes. When kinases go awry, diseases are often the result—especially cancer. For this reason, the kinase superfamily, with PKA as prototype, is a prominent target for drug therapy, and several effective anti-cancer drugs that work by blocking the active site of defective kinases are already available.

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To advance this work, Taylor and post-doctoral researcher Chris McClendon used Anton to simulate several different states of PKA’s catalytic domain, which binds with ATP and releases ADP. NMR studies by Gianluigi Veglia at the University of Minnesota, in collaboration with the Taylor lab, showed that these processes occur on slow biophysical timescales of milliseconds. With availability of Anton, McClendon was, for the first time, able to glean useful information from MD simulations about these cyclical structural changes.

A key finding was that the tail of the C-lobe, at the top of the PKA structure, flips open and closed like a latch to hold and release a “glycine-rich loop” that closes over the ATP. The simulations also suggest that active-site opening and closing can occur at rates faster than expected from prior studies. “We’re getting new clues as to what regions are dynamic,” says Taylor. “It’s the first time we can do calculations at a timescale we can experimentally validate. Anton provides us with a way to test the consequences of disease mutations and engineered mutations on the overall dynamics, which we have never been able to do.”

More info: www.psc.edu/science/2012/antonepics/