

Structure of the glutamate receptor's ligand binding domain, highlighting residues (red) that make connection across the cleft. The central residue forms a bond within the binding domain, stabilizing and locking the domain in the closed state.

Maria Kurnikova, left

Tatyana Mamonova, right



OPENING THE GLUTAMATE GATE

Simulations of the most prevalent receptor in the brain provide new understanding of how it opens its "gate" to fire neurons

In the time it takes you to read this sentence, more than a million neurons will transmit electrical impulses in your nervous system — triggered by touching this page, or using your eyes to make sense of the letters and words. These impulses — set off by touch, sight, sound and other stimuli — are relayed neuron-to-neuron through the brain by tiny messenger molecules called *neurotransmitters*.

You could think of a neurotransmitter as a lightning-fast pony express rider leaving St. Joseph, Missouri to get to Elwood, Kansas, the next stop, in microseconds. The distance between the two towns is like the synapse, the gap between a sending and receiving neuron. When the rider gets to Elwood, he hands his mail pouch to the next rider, whom you could think of as a receptor, a specialized protein on the membrane of the message-receiving neuron. As a neurotransmitter docks at a receptor, the neuron becomes activated, signaling it to release neurotransmitters for relay to the next neuron. But the interaction between the neurotransmitter and receptor must be just right, or the message won't be relayed, the next rider won't leave Elwood to journey across the next synapse.

It's a crude, imperfect analogy for an extremely complicated process. Though

incompletely understood, the complex interactions between neurotransmitters and receptors are the basis for the therapeutic action of many drugs. Prozac and its derivatives, for instance, treat clinical depression by binding to receptors for the neurotransmitter serotonin, increasing the amount of serotonin available in synapses.

Computational chemist Maria Kurnikova of Carnegie Mellon University uses supercomputing to study membrane proteins such as receptors and how they interact with neurotransmitters. In extensive work over the past three years, she and post-doctoral fellow Tatyana Mamonova used PSC's LeMieux and BigBen to gain new understanding of the receptor for an important neurotransmitter called glutamate.

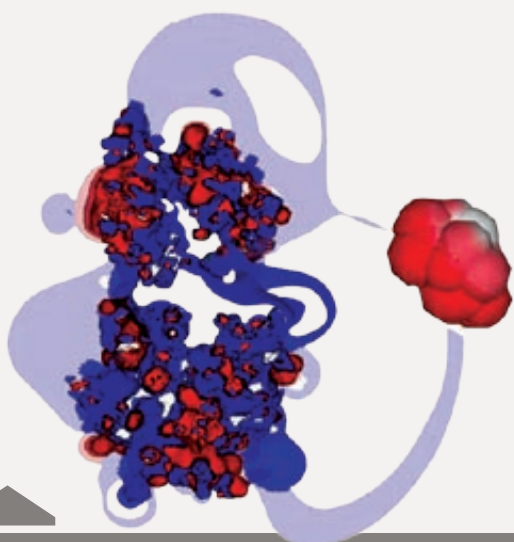
"What we found is exciting," says Kurnikova, "because it's something nobody expected." Their findings go beyond laboratory experiments and identify the precise atom-by-atom changes that occur as glutamate binds to the glutamate receptor, a process that changes the receptor's shape and eventually leads to activation of the neuron — information that presents new possibilities for precisely designed therapeutic drugs.

THE GLUTAMATE RECEPTOR'S FLYTRAP

Although glutamate may not be as well known to most people as serotonin and some other neurotransmitters, it's the most prevalent neurotransmitter in the central nervous system and an important focus of medical and pharmaceutical research. The interaction between glutamate and glutamate receptors plays an essential role in memory and learning, and dysfunction of this interaction is related to a diverse list of central nervous system disorders, including Alzheimer's disease, epilepsy, schizophrenia and depression.

A key to better understanding of all these dysfunctions is precise knowledge of how the glutamate receptor works. While there are several different glutamate receptors, Kurnikova and Mamonova looked at one (known as AMPA) that is the most common and initiates the series of steps that lead to neural activation. AMPA is a four-part protein (a tetramer) that forms a pore-like "ion channel" in the neural membrane — like a valve that opens selectively to allow ions, biochemical electricity, to flow through the channel into the cell.

The valve-apparatus of the receptor — its "gating mechanism" — that causes it to shift from closed to open was in large part a mystery when Kurnikova and Mamonova began their work. What was known is that each of the four parts of the receptor has its own "binding domain" — a cleft-like structure that extends outside the membrane — where glutamate can attach. When glutamate binds, this cleft in the binding domain closes — like a Venus flytrap catching a fly — which in turn causes the transmembrane channel to open for ion flow.



This image shows electrostatic charge, positive (blue) and negative (red), of the protein and ligand, glutamate (right), illustrating electrostatic complementarity between the ligand and protein.

"Cleft closure controls opening of the pore," says Kurnikova. "And the degree of cleft closure is believed to relate to how much the pore opens. Experiments show that this seems to be correct. But exactly how it happens nobody knows. It would be really nice to know on the molecular level what controls the degree of cleft closure, but to separate these processes into two — docked glutamate first and then the closed binding domain — is experimentally impossible."

REVEALING NEW DETAILS

Kurnikova and Mamonova tackled this problem by, in effect, building an atom-by-atom model of glutamate and the glutamate receptor binding-domain inside the computer. They used their model, comprising 20,000 atoms, with PSC systems — initially LeMieux, PSC's terascale system, and more recently BigBen — to simulate the shift from the unbound to bound state of the receptor.

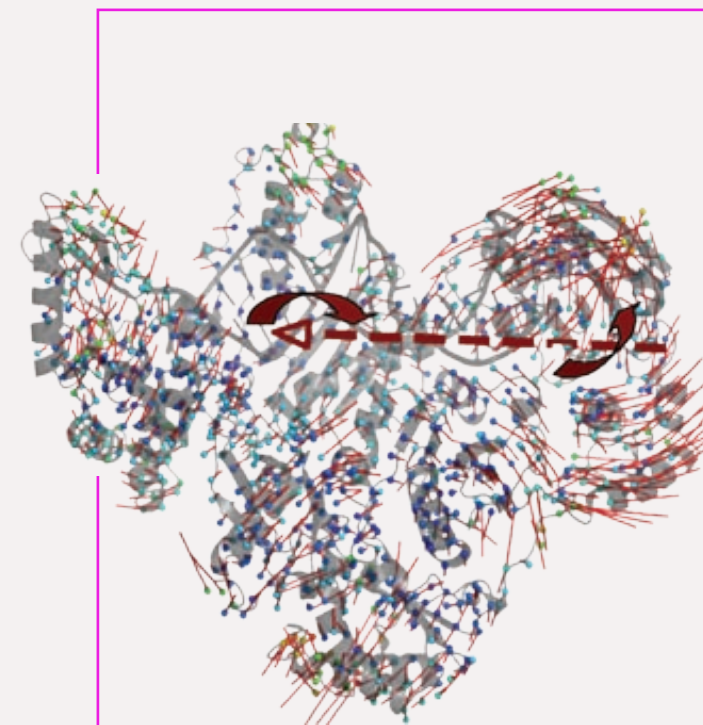
Using a method called "molecular dynamics," they tracked the forces among all the atoms as the receptor atoms shift position. They relied on software called AMBER (Assisted Model Building with Energy Refinement) and a special technique called "umbrella sampling."

In terms of the number of atoms, their problem was relatively modest. Their major challenge was to simulate the entire duration of the transition from unbound to bound state. With umbrella sampling, they divided the transition into 12 windows, each of which they simulated for 10 nanoseconds, a relatively long period in terms of biomolecular events. The simulations sliced time into femtoseconds, a million freeze-frame snapshots for each nanosecond. At each snapshot interval the simulation recalculated the forces and positions of every atom.

"Forcing a protein from one conformation into another is a challenging simulation," says Kurnikova. "Umbrella sampling is a technique where you bias the system to move slowly through its conformational space so you can sample the entire space. With mathematical techniques, you eliminate the biasing effects and calculate the free energy difference."

With their first series of simulations in 2004, the researchers compared results from their modeling to experimental data, gathered from infrared spectroscopy, measuring shifts in the vibrational frequencies of glutamate as it binds to the receptor. Their calculations closely reproduced the experimental results, validating their approach.

These simulations also provided insight into the positive/negative electronic polarities between the unbound receptor binding-domain — the open cleft



↑ This image from the simulation illustrates the axis along which RT seems to twist, a motion, says Madrid, that might allow the DNA to move across the protein and facilitate its replication.

Marcela Madrid



TWIST AND SLIDE: NEW CLUES TO KNOCKING OUT AIDS

In another project, PSC scientist Marcela Madrid collaborated with Maria Kurnikova and colleagues in a series of large-scale simulations of HIV-1 reverse transcriptase (RT). This multi-functional protein plays a critical role in the life-cycle of HIV, the virus that causes AIDS. It replicates HIV's DNA, essentially a copy-and-paste function, which is then incorporated into immune-system cells of the infected person. Because of this critical role, RT is the target of several FDA-approved anti-AIDS drugs.

Using PSC's LeMieux and the Cray XT3, Madrid and the Carnegie Mellon scientists simulated RT with and without DNA, simulations that involved 123,000 atoms. Notably, they extended their molecular dynamics simulation for 40 nanoseconds of biological time, much longer than any previous similar work. Because of this extended time, the simulations reveal motions and detailed interactions of DNA bound to RT not before observed. In particular, they show that the DNA undergoes a "twist and slide" motion, which may facilitate its positioning at RT's active site. Interfering with this motion could disrupt RT's function. "This work is important in understanding RT's function," says Madrid, "because it shows details of the motion that have not been observed before by any other computational technique."

of the Venus flytrap — and glutamate. They showed that the open cleft projects a positive electronic charge that strongly attracts the negatively charged molecular face of glutamate.

With the 2005 arrival of BigBen at PSC, the researchers were able to step up the pace of their work. "When we started," says Kurnikova, "running a simulation of one nanosecond would take several days. With BigBen, we were suddenly able to run 10 nanoseconds overnight."

The improved technology allowed them to do extensive simulations that tracked variant (mutant) glutamate receptor structures and the effect of the mutations on the transition from open to closed state of the glutamate binding domain. Ultimately, this allowed Kurnikova and Mamonova to identify precisely which molecular interactions controlled the binding-domain transition from open to closed. They identified several key interactions, but — most interesting — they found a bond that forms within the binding-domain itself once the cleft closes that, more than other interactions, stabilizes and locks the binding domain in the closed state.

"With BigBen, we were suddenly able to run 10 nanoseconds overnight."

"Nobody expected this from experimental analysis," says Kurnikova, "that there is this one interaction which is the most important and controls that whole transition." For drug design, this detailed knowledge is very important, she adds, and can guide pharmaceutical companies toward finding compounds with very specific effects — to either inhibit or enhance the gating mechanism of the glutamate receptor.

"That's the advantage of atomistic simulations," she says. "We can look at that experimentally and observe some effect and guess why it works, but with this modeling we can see what causes what we see experimentally — not just what happens, but how. We can understand that in great detail." (SP)

MORE INFORMATION

<http://www.psc.edu/science/2008/glutamate.html>