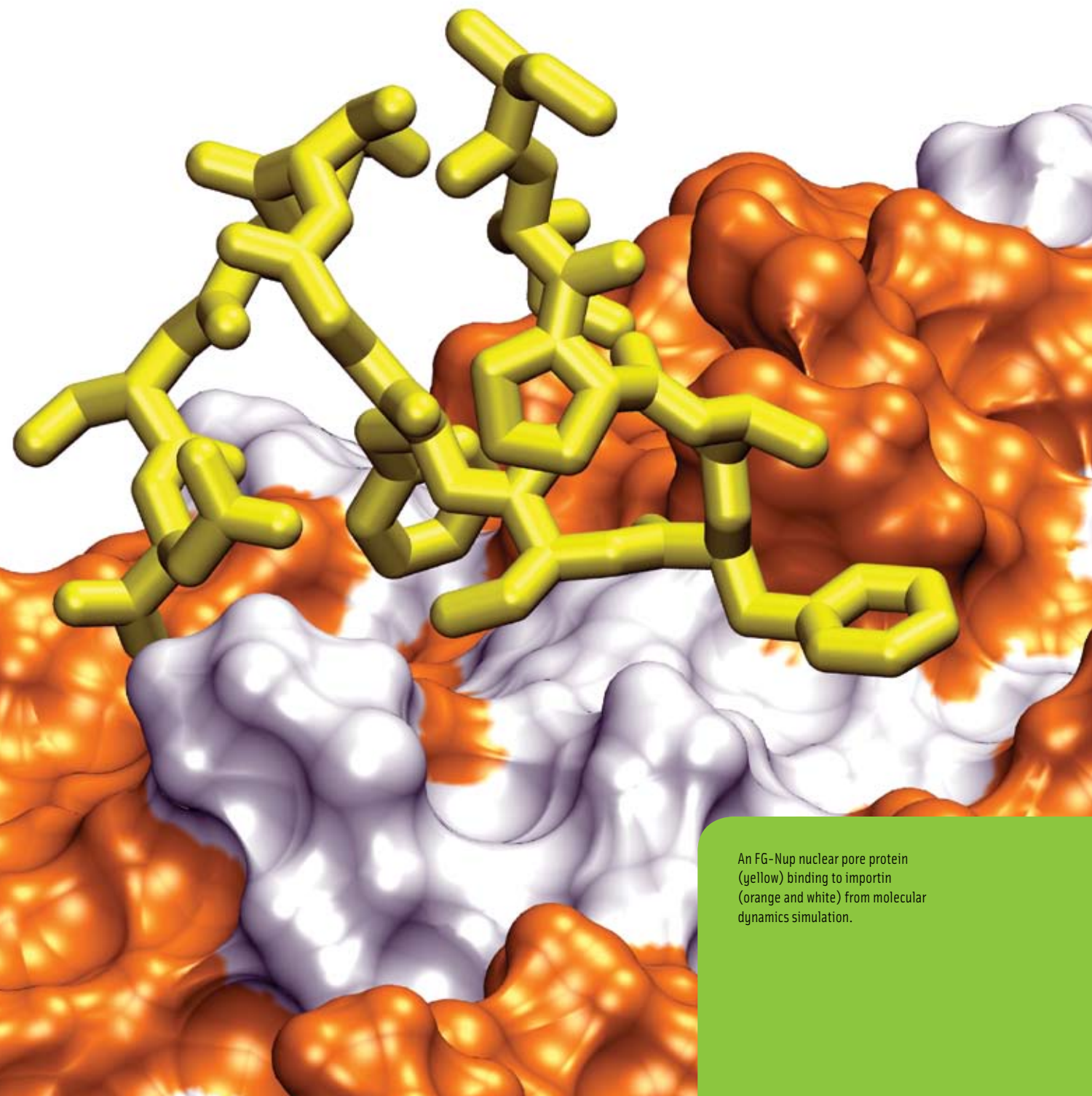


KNOCK, KNOCK, WHO'S THERE?



An FG-Nup nuclear pore protein (yellow) binding to importin (orange and white) from molecular dynamics simulation.

SIMULATIONS REVEAL NEW INFORMATION ABOUT THE GATEWAY TO THE CELL NUCLEUS

Think of yourself as a big protein. You're inside a cell, milling around in the aqueous molecular crowd called the cytoplasm, and you'd like to get into the nucleus. If you've ever attended a black-tie event with someone at the door to check your engraved invitation and escort you arm-in-arm through the doorway, where you can pass only if the escort displays correct credentials to a very serious bouncer — that's a rough idea of what it's like to pass into or out of the cell nucleus.

The doorway with built-in security apparatus you'd have to go through is called the nuclear pore complex. Hundreds to thousands of NPCs are embedded in the nuclear envelope of each cell, a double membrane that houses and protects the organism's genetic information. The NPC's ability to pick and choose which large proteins pass is crucial, protecting the nuclear DNA and, at the same time, allowing transit of proteins and strands of RNA involved in the manufacture of new proteins, the cell's bottom-line business.

Small proteins pass freely, but for a large protein to pass through the NPC may be only slightly less complicated than the proverbial rich man getting to heaven. "The mechanism by which the NPC selectively allows the transit of certain large proteins while restricting the passage of others is poorly understood," says biophysicist Klaus Schulten, who directs the Theoretical Computational Biophysics Group at the University of Illinois Beckman Institute.

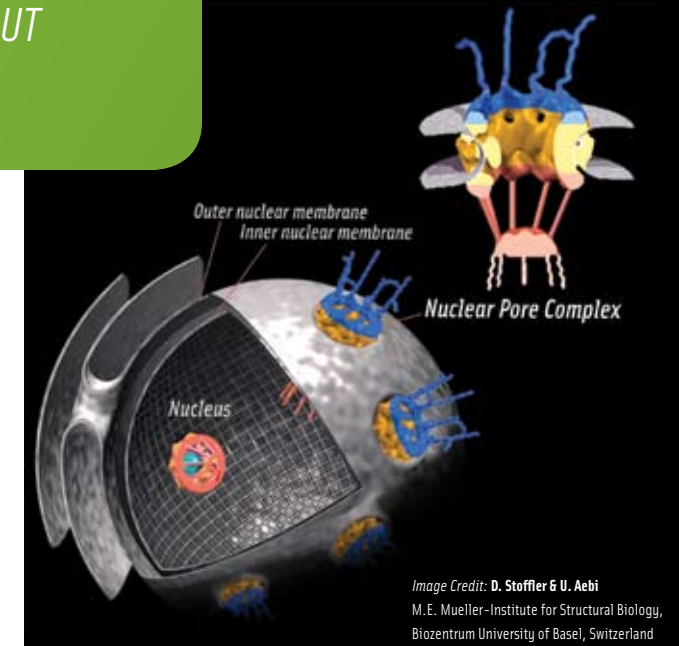


Image Credit: D. Stoffler & U. Aebi
M. E. Mueller-Institute for Structural Biology,
Biozentrum University of Basel, Switzerland

NUCLEAR PORE COMPLEX

The NPC's selection process involves signal proteins, energy-transfer reactions and it relies strongly on a group of proteins — called transport receptors — whose job is to escort large proteins through the NPC. In a late 2005 study, Schulten and graduate student Timothy Isgro looked at a transport receptor called importin- β and how it interacts with other proteins in the NPC interior. Their molecular dynamics (MD) simulations — using TeraGrid resources at PSC, SDSC and NCSA — provide the first atomic-level view of NPC selectivity, and they show that these interactions are more numerous than had been thought.

Their work confirms experimental findings about importin and goes beyond experiments to predict new "binding spots," one of which has been subsequently verified. Schulten and Isgro's ability to reveal previously unknown details of the NPC process with simulations attracted attention, including commentary in *Science* (January 6, 2006) and *Structure* (December 2005), which stated "the predictive power of their MD work is convincingly displayed...."



Klaus Schulten (left) and Tim Isgro,
University of Illinois, Urbana-Champaign



ONWARD TO THE RIBOSOME

The life sciences, says Klaus Schulten, are moving on to bigger things. When it comes to understanding processes in living systems at the molecular level, it's become possible—thanks to technology such as the Cray XT3—to look at very large systems, of which the nuclear pore is one example. “The XT3,” says Schulten, “is a terrific computer platform for this new generation of work.”

Encouraged by success with the NPC, Schulten has (as of late August 2006) begun to use his powerful software NAMD on PSC's XT3 to simulate the ribosome. This huge aggregate protein-RNA assembly is a cellular factory that reads the genetic blueprint from messenger RNA and uses it to manufacture proteins. “It's possible to do this now with the XT3,” Schulten's MD simulations of the ribosome (2.7 million atoms) are using NAMD with 1,000 XT3 processors—unprecedented large-scale parallelism for molecular dynamics.

Because of the XT3's fast inter-processor communication, NAMD can use the entire system efficiently, 2,000 processors, for a single simulation. “This is just the right system for this kind of project,” says Schulten. “If you have a tennis racquet, it has a sweet spot where you can hit best, and for this project the sweet spot is the entire XT3 at Pittsburgh.”

GATEWAY TO THE NUCLEUS

In the world of proteins, the NPC is a behemoth. Its mass in vertebrates is 125 megaDaltons, probably the largest protein complex in the cell. Not a single protein, the NPC is comprised of many distinct proteins, called nucleoporins — “Nups” for short. “There are whole machines in living cells that are made of hundreds or thousands of proteins,” says Schulten, “and the nuclear pore is one of those systems. It's actually one of the most magnificent systems in the cell.”

The immense size of the NPC makes it difficult to study in detail. Over the past decade, electron microscopy has provided much information about its large-scale structure, and other laboratory studies have looked at the transport process. Recent work resolved the structure of importin, laying a foundation for Schulten and Isgro's MD simulations. “The mystery,” says Isgro, “is how exactly does the nuclear pore complex transport things into and out of the cell nucleus.”

Experiments have shown that a specific subset of Nups — called “FG-Nups,” because of a repeating sequence of the amino acids phenylalanine and glycine — is involved in NPC selectivity. “Presumably the NPC recognizes importin as a protein that's allowed to pass and shuttle its cargo through the pore,” says Isgro. “The question is what is the recognition process? Experiments have implicated the FG-Nups.”

FG-Nups have been shown to occupy the central pore region of the NPC, through which a transport receptor and its cargo must pass, and they have been shown to interact with importin at binding sites on the importin surface. To get a detailed picture, Schulten and Isgro turned to MD simulations, which track how the atoms move with time, and can give a highly detailed movie of protein interactions.

PREVIOUSLY UNKNOWN SITES

As a first step, Isgro used PSC's LeMieux to simulate FG-Nups alone, without importin. The question was whether it would be feasible to see results from a simulation of importin with FG-Nups in the amount of biological time it would be possible to simulate — tens of nanoseconds (one nanosecond is a billionth of a second) being the limit with a practical amount of computing.

“I didn't know,” says Isgro, “if tens of nanoseconds would be enough time for the FG-Nups to diffuse through solution and bind to the surface of importin.” Using MD software called NAMD, developed in Schulten's research group, with 128 processors of LeMieux for a series of sixteen 24-hour simulations, Isgro found that the FG-Nups associated with one another in a few nanoseconds, which suggested that a simulation with importin would be fruitful.

“LeMieux,” says Isgro, “provided a fast and efficient computing platform that enabled us to see the full atomic structures and dynamics of nuclear pore proteins interacting with one another for the first time.”

To simulate the interaction of importin with FG-Nups, Schulten and Isgro also used NAMD and a system at NCSA. They did five different simulations, using different FG-Nup sequences in solution with importin. They simulated a total of over 200 nanoseconds, each simulation lasting several tens of nanoseconds, enough time to see FG-Nups bind to the surface of importin.

THEY IDENTIFIED SIX PREVIOUSLY UNKNOWN BINDING SPOTS.

As a further step, the researchers aligned the amino-acid sequences of eight different forms of importin to identify likely “hot spots” for FG-Nup binding. With this combination of MD simulations and sequence alignment, their computations confirmed three out of four FG-Nup binding sites on importin that had been previously identified in experiments. Their study also identified six previously unknown binding spots.

An experimental study subsequently verified one of these newly predicted binding spots. These additional binding spots, says Schulten, suggest that importin has more extensive ability to interact with the NPC than had been expected before this work. The observed interactions between FG-Nups and importin are consistent with several prevalent models of how the NPC recognizes transport receptors and allows them to pass. “These results,” says Schulten, “are the first atomic-scale look at these novel binding spots for FG-Nups, and they suggest that the extent of binding on the surface of importin is much larger than previously realized.”

With this success under their belt, Schulten and Isgro in 2006 turned to another transport receptor, NTF2 (nuclear transport factor 2). As they did with importin, they looked at the interactions between NTF2 and FG-Nups. For this project, PSC's Cray XT3 was available to speed up completion of the work. Once again, the researchers found previously unknown FG-Nup binding spots on the transport receptor, and the simulations provide previously unavailable structural detail of the binding interactions. (MS)

MORE INFORMATION:

<http://www.psc.edu/science/2006/schulten.html>

FG-Nups in Solution

A snapshot from simulation on PSC's LeMieux shows FG-Nups (various color strands) interacting with one another. This is a hypothesized view of the center of the nuclear pore complex, in which the disordered FG-Nups would block large proteins, yet allow passage of transport receptors, such as importin, with their large protein cargo. Phenylalanine residues (the “F” of “FG”) are displayed in blue (hexagons). Ions are shown as spheres.

