

BENT 'BRANES

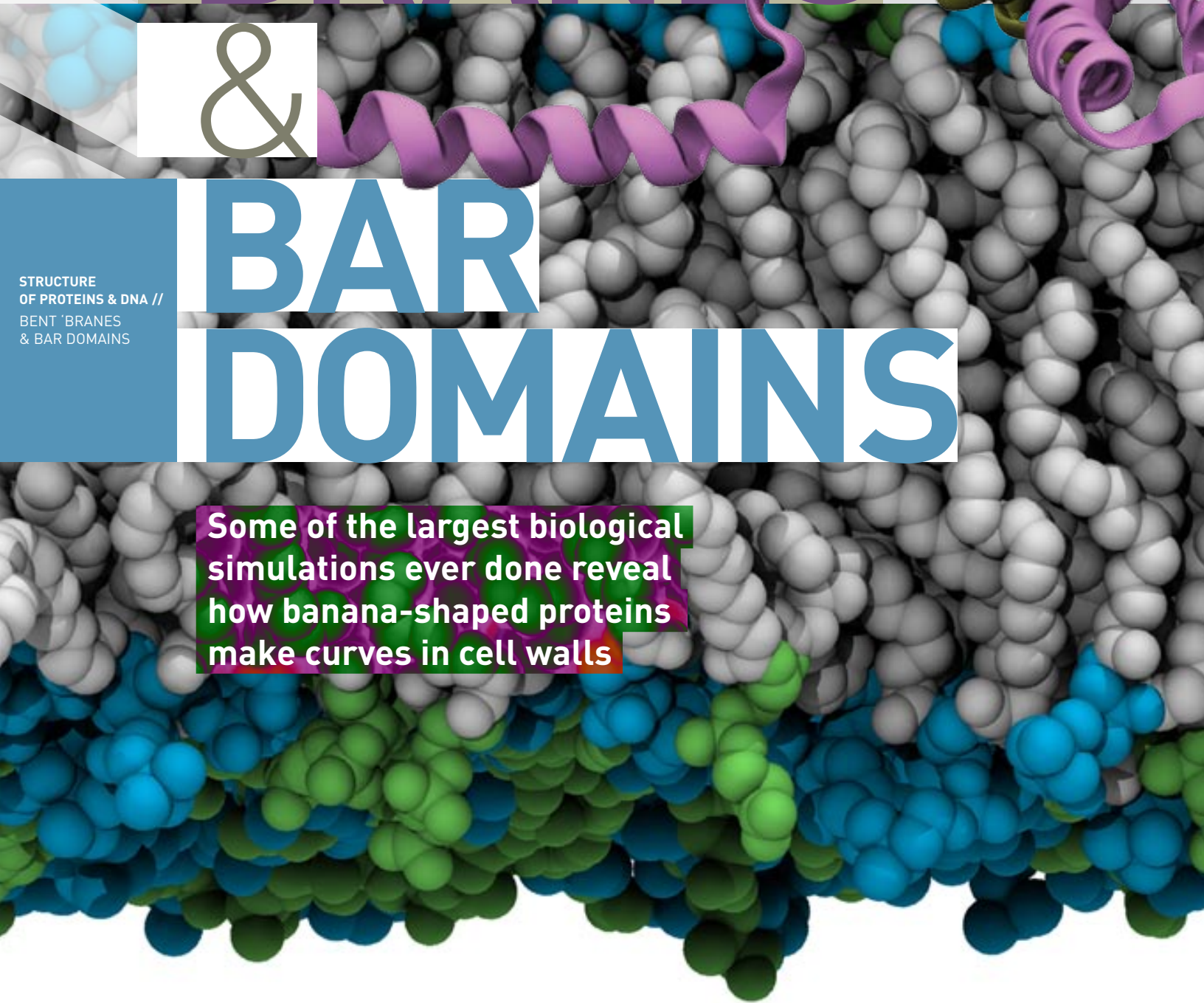
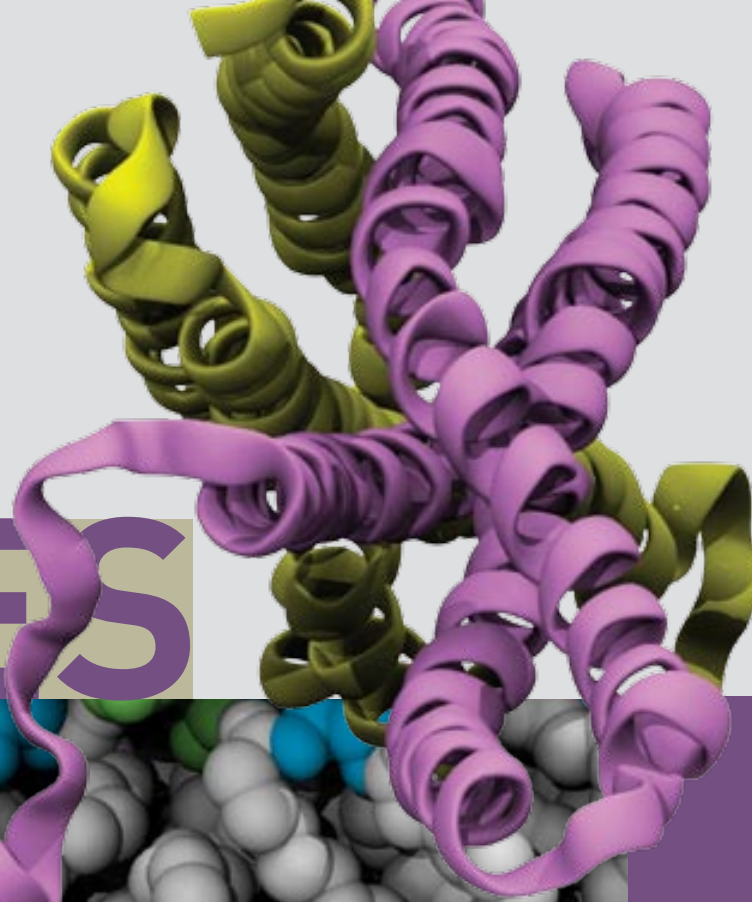


BAR

DOMAINS

STRUCTURE OF PROTEINS & DNA // BENT 'BRANES & BAR DOMAINS

Some of the largest biological simulations ever done reveal how banana-shaped proteins make curves in cell walls



Gregory Voth



Phil Blood

Think of "The Blob" in that bad old movie — a giant amoeba-like alien that globs onto and absorbs humans. Endocytosis, the life-sustaining process by which cells absorb large proteins and other molecules, is something like that, and it's an amazing ability. A squishy, water-resistant wall that isolates and protects the cell's innards, the cell membrane can also pucker into a mouth-like opening that engulfs external molecules and closes off into a spherical chamber called a "vesicle" that's absorbed into the cell.

Similar abilities of the cell wall to form a vesicle are involved in transporting molecules out of the cell, such as neurotransmitters bursting out of nerve cells. Other processes, such as those that generate tubules in muscle cells, also involve curving of the cell wall. All these processes have many steps, but they all start with "remodeling" — a change in membrane curvature.

"Membrane remodeling is one of the most important phenomena in cellular biology," says University of Utah chemist Gregory Voth. "It's fundamental to vesicle budding, a key feature of many processes, one of the most well known being neurotransmitter release in the synapses."

Experiments show that these complex events are triggered by an ensemble of proteins, and they show also that a banana-shaped protein segment called the BAR domain is a key player in inducing the cellular membrane to curve. But how? The experiments show that the BAR domain is involved, but they don't show the structural and dynamic bottom-line, what individual atoms are doing as curvature proceeds, which is where Voth and Phil Blood — then a University of Utah Ph.D. candidate, now a PSC scientist — came into the picture. "What we've been able

to do via molecular dynamics simulations," says Blood, "is to see the atom-by-atom details of how these proteins generate membrane curvature."

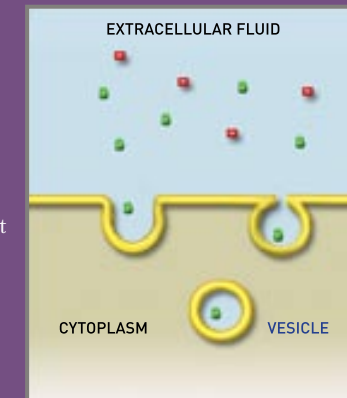
Using PSC's Cray XT3 as their workhorse, along with TeraGrid systems at SDSC, NCSA and the University of Chicago/Argonne, Voth and Blood did molecular dynamics (MD) simulations that track the atom-level interactions between a BAR domain and a model of cellular membrane.

Their simulations — involving 738,000 atoms, using more than two-million XT3 processor hours — are among the largest biological computations ever performed.

Their results, reported in *Proceedings of the National Academy of Sciences* (October 10, 2006), confirm and go beyond experimental results, showing that BAR domains act as a scaffold, forcing the membrane to adopt their curvature, and that the orientation of the BAR domain as it attaches to the membrane determines the degree of curvature.

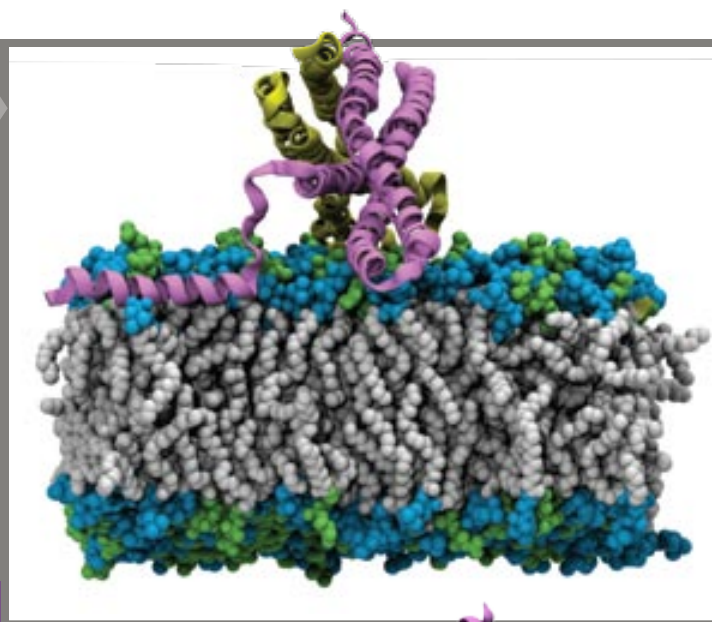
Extending this success from 2006, Voth and Blood this year refined their simulations to look more deeply and see more exactly which parts of the BAR domain structure drive curvature. They found — contrary to what had been surmised from experiments — that helical structures at each end of the BAR domain do not by themselves initiate curvature.

"With this kind of work," says Voth, "we're beginning to see how the modern capabilities of molecular dynamics simulation touch very fundamentally important biological processes. There's been this investment both in hardware and software. This combination is paying off. With this kind of problem, we're getting at the interplay between proteins and membranes at a level beyond binding studies and ion-channel transport, and we're able to see whole-scale changes in the membrane morphology driven by these interactions."

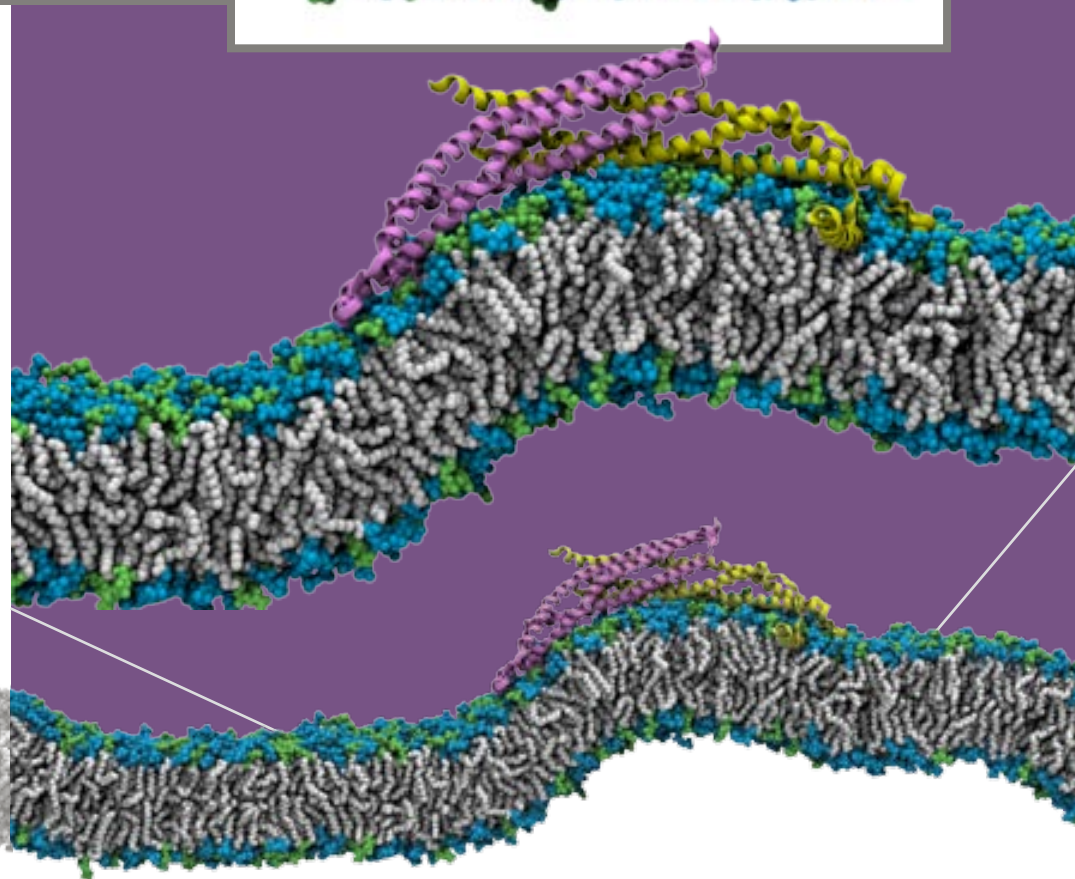


Bending the Membrane

This front view (below) from a simulation on PSC's Cray XT3 represents a BAR domain protein segment (pink & yellow helices) bending a segment of cellular membrane with negatively-charged lipid head groups (green) after 30 nanoseconds of simulation. Loops on each end of the BAR domain bind tightly to the descending sides of the curved membrane. The N-terminal helix (yellow) is embedded in the bilayer. The side view (right) shows a cutaway of the embedded N-terminal helix and also shows the loop structure that binds to the membrane surface.



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TOOLS FOR THE JOB

In 2004 a team of British scientists laid the groundwork for Voth and Blood's simulations by working out the three-dimensional structure of the BAR domain, work that appeared on the cover of *Science*. "They solved the crystal structure for this protein," says Blood, "and it was quite interesting to see that it looks like a banana, a crescent shape that immediately says to you, 'I'm a curvature forming protein.'"

Beginning with its shape, the BAR domain suggested a number of possibilities for how it could generate curvature. The concave side of the crescent carries a positive electronic charge, which could bind to the negatively-charged surface of a cellular membrane and mold it to a matching curvature.

BAR domains also have a helix structure at each end, and experiments suggest that these "N-terminal helices"

insert into the membrane, anchoring the protein at its base on both ends, which could help to induce puckering of the membrane. "There are many nuances," says Blood, "but nothing had been shown explicitly."

As Blood began to build a computational model, using the DataStar at SDSC and IA64 at NCSA along with a system at Argonne, he knew he'd taken on a demanding task. "The TeraGrid has been essential to making this possible." With MD software called NAMD (Not just Any Molecular Dynamics program), developed by Klaus Schulten's group at the University of Illinois, Urbana-Champaign, along with the XT3, Blood was working with the most powerful tools available for MD simulations at the large scale his problem demanded. "NAMD has been amazing," he says, "because it scales well even with the long-range electrostatics."

The problem required accurate treatment of the electronic charges between atoms, and the MD software tracks these forces as they vary between every atom in the simulation at each time step as the simulation progresses.

Scaling, a crucial feature for a large problem such as this, means that as you recruit a large number of processors for the computation — Blood used 512 XT3 processors — the per-processor performance remains high, doesn't take a prohibitive hit due to the inter-processor communication involved in tracking the atom-to-atom dynamics.

Among MD programs, NAMD is exceptional in its ability to run efficiently with hundreds or thousands of processors, and this is especially true on the 4,000-processor XT3, which has outstanding inter-processor bandwidth. "With the XT3, we haven't found a hard upper limit on scaling," says Blood. "Our simulations have run well with 512 processors, and we've used up to 1,024 processors on this system with little dropoff."

AT THE FRONTIER

As Blood built his computational model and explored the size of the problem, he began with a patch of membrane — 342,000 atoms, 25 nanometers in length — that was in itself a demanding computation. Trial studies showed, however, that this wasn't enough length for curvature to become apparent. Not long after the XT3 became available as a production system in October 2004, Blood more than doubled the size of his membrane model to 738,000 atoms — a 50-nanometer length of membrane, the longest patch of membrane ever simulated.

"The XT3," says Blood, "came online just at the time when we needed it to make this work."

He ran two very large-scale simulations with this membrane and the BAR domain, tracking 27 nanoseconds (billionths of a second) of the dynamics, at time steps of two femtoseconds (millionths of a nanosecond). The two simulations resulted in two different orientations of the BAR domain, with the membrane curving to match the protein in each case. A control simulation with the membrane alone, no protein — on the DataStar system at SDSC — produced no similar curvature.

THIS IS THE FRONTIER IN THIS FIELD. THESE SIMULATIONS ARE INDISPENSABLE.

These results showed convincingly that the BAR domain generates membrane curvature and that the degree of curvature depends on the BAR domain's orientation. For Blood, they also raised a question. "How much of that curvature is due to the N-terminal helices and how much is due to the charged concave surface?"

Some experiments suggested that the helices were the main driving force, with the concave surface providing little more than a scaffold. Another series of simulations, mainly on the XT3, showed that — at least for an individual BAR domain — this is not the case. These simulations showed, furthermore, that a particular structural element of the BAR domain, positively charged loops near the end, are crucial, binding to and stabilizing the BAR domain on the membrane surface, and without the binding of these loops curvature doesn't occur. "From these studies," says Blood, "it looks like the N-terminal helices are not primarily responsible for driving curvature."

In a follow-on study (*Biophysical Journal*, May 2007) Voth, Blood and their colleague Gary Ayton used the MD results as input to a larger-scale model of membrane shape change. "We use the molecular dynamics to feed a model at the meso-scale," says Voth, "and show how the interactions generated at the atomistic level propagate and scale up to a larger scale of remodeling."

Based on their success with NAMD and the XT3, Voth and Blood are looking ahead to even bigger, more realistic simulations. "The next generation of these simulations," says Voth, "will push up to 10-million atoms, which we'll need to look at multiple interacting BAR domain proteins and to develop accurate but simpler coarse-grained models for these systems. In nature, it's the collective behavior of many of these proteins interacting with the membrane that creates curvature.

There isn't a good experimental way to look at this. This is the frontier in this field. These simulations are indispensable."

MORE INFORMATION:
www.psc.edu/science/2007/bardomain.html