Physicists Advance Into Biology

Physicists are bringing their world view to bear on biological phenomena ranging from cell membranes to cell movement, hoping that their mechanistic approach will yield new insight into biological systems

ST. LOUIS—On a blustery March day during a recent scientific meeting here, Igor Vodyanoy walked to lunch while joining in a cheerful conversation about the successes of molecular biology. Then someone suggested that the standard picture of how cells work needs only the finishing touches; the basics can already be found in textbook descriptions of proteins that carry out specific functions depending on their structure. Vodyanoy stopped in his tracks and raised a monitory finger. "That does not tell you the mechanism" of how everything from molecules to membranes interacts, he said. "For that, you need to know the physics."

Vodyanoy, of the London office of the U.S. Office of Naval Research, and colleagues were at the meeting of the American Physical Society, where the idea that biology is sorely in need of "physicist" thinking was a popular one. In a number of sessions devoted to biophysics, dozens of papers—given by researchers with both physics and biology backgrounds—argued that biology needs universal, mechanistic laws and detailed predictions, the hallmarks of physics. For example, several presenters argued that the usual modus operandi in biochemistry—precise determination of a molecule's structure followed by qualitative reasoning about its function—all too often misses the subtleties of how molecules interact. Eventually, says biophysicist Evan Evans of the University of British Columbia in Vancouver, "we're going to know almost every protein structure we need, and yet we're not going to know how things work."

Biologists in and outside of the meeting counter that an understanding of structure is the first vital step, without which any further analysis will run aground. And finding universal laws of life is easier said than done. Indeed, because physicists have a certain naiveté about the complexity of biological systems, they tend to issue narrow predictions that may not be biologically relevant, says cell biologist and cancer researcher Andrew Maniotis at Harvard Medical School. Yet Maniotis and others agree that biology could benefit from a focus on mechanism. The new attention by physicists is "greatly welcomed," says structural biologist and biophysicist Walter Mangel of Brookhaven National Laboratory. At the meeting, the biophysicists were eager to show off what they have to offer, demonstrating how a physicist's point of view can yield new insight into a range of biological systems, from the dynamics of receptors on cell membranes to the diffusion laws governing the migration of cells during development.

Researchers don't always agree on precisely what distinguishes the Weltanschauungen of the two disciplines, beyond noting that physicists tend to strive for simplicity and quantitative predictions, while biologists are more intent on synthesizing many interrelated phenomena in order to understand complex systems. But recognizing the difference is as easy as knowing hawk from handsaw when you see them, the researchers say. That's the case with new theoretical work on how cells adhere to each other, in which the physicist's handsaw is powerfully evident.

The work, presented at the meeting by Robijn Bruinsma of the University of California, Los Angeles, and done in collaboration with Daniel Zuckerman of the University of Maryland, College Park, focuses on the interactions between the so-called "lock-and-key" molecules on cell membranes. These molecules allow certain cells to stick together, an ability central to the immune response, normal tissue development, cancer metastasis, and other physiological processes.

ENRICO DI CERA ET AL.

Shape shifter. Thrombin changes its whole conformation in response to the binding of a single ion.

For example, cell membrane dynamics can have a "dramatic" effect on how a killer T cell binds to a macrophage, says Bruinsma. This binding takes place after the macrophage, acting as a sort of roving garbage truck, engulfs bacteria and expresses specific molecules (the "keys") on its surface. Once the keys are displayed, they fit into "locks" on the T cell, signaling it to release a chemical that destroys the macrophage's contents, Bruinsma explains. But the short-range attractive forces, or "stickiness," generated by these lock-and-key molecules are balanced by a long-range repulsion, caused by hairy glycoprotein molecules that hang from the cells and resist being compressed.

So how strongly can the T cell bind to the macrophage, given the opposing forces and the finite number of receptors? Earlier treatments had considered the lock-and-key molecules as a uniformly distributed "adhesive," but Bruinsma and Zuckerman note that the molecules can slide along the membrane surfaces. As the glycoproteins push the cells apart, they create "dimples" where the membranes bow apart, and the lock-and-key molecules tend to slide together at the edges of these dimples, where the membranes are closest.

The team found that, mathematically, the lock-and-key molecules behave as a peculiar kind of two-dimensional "plasma," or ionized gas, in which every particle has an apparent attraction to every other particle. The resulting collapse of molecules to the dimple edges "gives [the cells] a foot to hold onto," creating a strong, ropelike bond that is more powerful than an array of individual connections, each of which could easily be peeled away, explains Evans. He notes that experiments could look for this effect in cells. "Biologists studied certain types of adhesion molecules as individual cases," says Bruinsma. "We looked for the generic features of adhesion and stumbled on a very interesting problem from the viewpoint of theoretical physics." It may be an important result for biologists too, as similar binding occurs in many kinds of infected cells. And certain diseases, such as cancer metastasis, occur when cells are not sticky enough, adds Cyrus Safinya, a biophysicist at the University of California, Santa Barbara.

Electric results

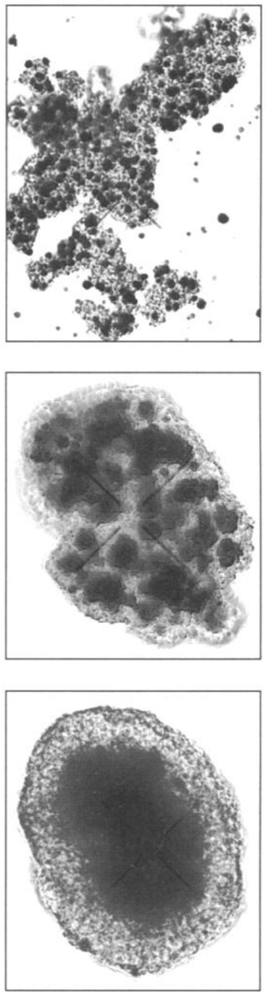
The physicist's Weltanschauung is providing new insight into other key roles of the cell membrane too. For example, at the meeting Vodyanoy presented work done with his collaborator Sergey Bezrukov of the National Institutes of Health in Bethesda, Maryland, focusing on proteins that conduct ions through membranes—the so-called ion channels that occur in virtually every living cell. These channels regulate everything from a cell's osmotic tension to the conduction of signals along nerves. The kind of channels studied by Bezrukov and Vodyanoy are called gated, because they open and close stochastically, or unpredictably, in response to electric fields applied across the membrane.

In some cases, these channels react to extraordinarily tiny changes in electric field. Sharks, for example, can sense the infinitesimal, periodic electric fields generated by the opening and closing of a prey's gills amid a much stronger background of electric noise, effectively "amplifying" only the prey's signal. Artificial devices can achieve such high performance only at liquid-nitrogen temperatures, says Vodyanoy. "And here we have this shark [simply] floating there," he says. "The channels [in its sensory organs] are somehow able to sense that minuscule field."

Bezrukov and Vodyanoy set out to understand the basis of that sensitivity, using a carefully controlled experimental model of one living system. In experiments reported late last year in *Nature*, they studied ion-channel proteins embedded in artificial membranes that separated reservoirs of electrolyte. They applied small periodic fields to their artificial membranes and found that the amplification of the signal—as estimated by the ion currents driven through the channels—increased nearly exponentially as they turned up the strength of a separate, noisy field. In other words, the more noise, the greater the membrane's ability to sense the field. This counterintuitive phenomenon, called stochastic resonance, has been seen in much more complicated biological systems, starting 2 years ago with pioneering work by Frank Moss of the University of Missouri, St. Louis, who studied the ability of crayfish tails to sense motion.

With the Bezrukov-Vodyanoy setup, however, "one can begin to do something really quantitative at the membrane level," says Moss—and perhaps eventually lay bare the molecular basis for the effect. At the meeting, Vodyanoy briefly described new computer simulations suggesting that the amplification depends on correlations between adjacent channels, and also on the gates' sensitivity to a wide range of fluctuating voltages. This reliance on the fine details of the noise could be a critical clue to the dynamics, the researchers say.

While Bezrukov and Vodyanoy explore cells' sensory abilities, other biophysicists are zeroing in on cell movement—specifically on the complex choreography of embryogenesis, when groups of cells migrate to their proper positions in the developing organism. This process is undergoing detailed scrutiny by a team at the University of Notre Dame, led by James Glazier and including Arpita Upadhyaya, Yi Jiang, and José Mombach (now at the Universidade Federal do Rio Grande do Sul in Brazil). At certain stages in the development of an embryo, cells are thought to execute "random walks"—they move in random directions until they find a state of minimum energy that is determined by how strongly different cells stick to each other when they happen to collide, explains Glazier. But until now no one has been able to follow the process in detail.



GLAZIER ET AL.

New order. During development of a chick embryo, pigmented eye cells diffuse randomly through mixtures of other cells to form a predictable shape.

"Everybody talks about Brownian motion"—cell diffusion in random directions—"but nobody tries to validate that quantitatively," says Glazier. His group has done just that, analyzing digitized, time-lapse VCR images of pigmented and neural retinal cells taken from chick embryos. They found that the darker, pigmented cells from the back of the eye indeed diffuse randomly through mixtures of neural cells, like molecules in a liquid. Theorists have proposed that this particular kind of "cell sorting" dominates at certain stages of embryonic development. Now Glazier hopes to create general models of development by including chemotaxis—a cell's tendency to move toward or away from a substance—as a second cell-sorting mechanism.

Glazier, a physicist noted for his work with bubbles and foams, concedes that doing physical experiments in biological systems can present unfamiliar obstacles. "Biology is a lot less obliging than physics," he says. "You can't just dial up a temperature or pressure," but instead must learn biologists' techniques for coaxing living cells to behave reproducibly.

Cooperation in action

But such issues present no problem for the leadoff speaker in a session devoted to cooperative effects in biological macromolecules: Enrico Di Cera of the Washington University School of Medicine in St. Louis, who has a biology background but has embraced a quantitative philosophy. Di Cera works with thrombin, a key enzyme in the cascade of events leading to blood coagulation in humans. He was dissatisfied with standard biochemical studies, which rely on a combination of structural probes and mutation experiments, because he believes they yield a "very superficial understanding" of structure-function relationships. For example, a particular mutation in a peptide chain might change its activity, suggesting a function for that part of the protein, but this doesn't reveal whether that site's behavior also reflects the influence of other sites on the molecule.

Such "cooperativity" is common in large molecules such as thrombin, where binding of other atoms and molecules at one site can trigger shape changes throughout the entire molecule and affect binding affinities at other sites. Indeed, one such shape change causes a radical shift in thrombin's function—from enhancing to inhibiting blood coagulation.

To understand these cooperative effects, Di Cera built on work by Gary Ackers of Washington University and others to develop a rigorous thermodynamical theory that links the binding energies at pairs of sites on a large molecule. Like the pressure and volume in an engine's cylinder, which depend both on each other and on a third parameter such as an imposed temperature, the binding energy at one site can depend both on the state (bound or unbound) of the second site and on conditions in the rest of the molecule. Di Cera has shown mathematically that by constructing such energy diagrams from measurements made at pairs of active sites in a molecule, one can unravel the exact nature of the sites' interactions.

Now he and several coworkers, including Quoc Dang, Enriqueta Guinto, and Alessandro Vindigni at the medical center, are applying the formalism to experiments on thrombin. They have found that the molecule's dramatic conformational change is controlled by the binding of a single sodium ion to a particular site on the molecule—a vivid instance of cooperativity between that site and the rest of the structure. Such work could advance the search for an anticoagulant that prevents heart attacks by blocking the active sites of thrombin, says Jules Shafer, director of basic research at the Merck Research Laboratories in West Point, Pennsylvania. But Shafer says that most biochemists haven't had time yet to warm up to the complicated general formalism.

Whether biologists finally welcome these new incursions into their field may depend on whether physicists make up for "broken promises" of earlier times, says James Lewis of Arizona State University, who spoke at a session on applying quantum mechanics to large biological molecules. Thirty years ago in his own specialty, says Lewis, "the speculation was that, 'Gee, we ought to be able to turn the crank on any biological system you can think of,'" solving problems of molecular dynamics, such as interactions of electron clouds, more precisely. Physicists' hopes didn't pan out, mainly because available computing power was too small, says Columbia University's Robert Friesner. But he and others argue that hardware and software advances now allow physics to make a major contribution to biology.

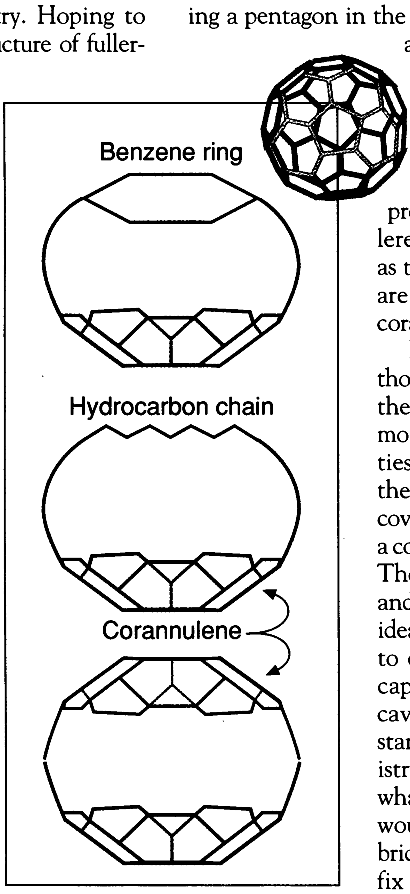
Whatever reception such ideas find in biology, they're spawning some interesting physics, says Philip Pincus, a collaborator of Bruinsma's at the University of California, Santa Barbara. "You pick up one of these big biochemistry books," he says, "and on nearly every page you find interesting physics questions that for the most part haven't been studied." In the end, says Pincus, physicists may profit from the wide-open new territory of biophysics at least as much as biologists.

–James Glanz

CHEMISTRY

Molecule Promises a Better Buckyball

Ever since the cagelike carbon molecules known as fullerenes were first made in usable quantities 5 years ago, chemists across the globe have been putting aside what they were doing and setting forth into this new territory of fullerene chemistry. Hoping to exploit the unique hollow structure of fullerenes to create new catalysts, sensors, and other chemical marvels, these pioneers have attached almost every known chemical group to the surface of "buckyballs." They have trapped metals inside them, punched holes in them, and made them glow by trapping them inside porous minerals.



Basketmaking. By bridging corannulene baskets, chemists hope to mimic the buckyball *(top)*.

But some of the bloom is off the buckyball, says Jay Siegel, a synthetic organic chemist at the University of California, San Diego, because it is hard to tailor buckyballs' chemical and physical properties for a particular use. The problem with buckyballs is that they are made in the flash of an electric arc, with no chance to manipulate their structure as they form. Siegel, however, has an alternative: what might be called buckybaskets.

Last month in the *Journal of the American Chemical Society* (*JACS*), Siegel, Jon Seiders, and Kim Baldridge describe how they synthesized buckyball-type molecules that should retain the advantages of the original, but whose size, shape, and overall chemistry may prove much easier to tailor. "Siegel's work appears to be a real breakthrough. Their approach ... promises to afford some very novel compounds," says chemist Peter Rabideau of Louisiana State University in Baton Rouge.

Siegel and his colleagues begán with a molecule called corannulene, which is shaped like a skullcap and can be thought of as a fragment of a buckyball. The basic corannulene molecule is essentially five hexagonal benzene molecules fused to form a ring, creating a pentagon in the middle. The molecule assumes a cap shape because a ring of five hexagons simply cannot lie flat when their edges are stuck together. "Some of the properties that make fullerenes interesting, such as their electrochemistry, are already apparent in corannulene," says Siegel.

He and his colleagues thought they might endow the molecule with even more interesting properties if they could close off the cavity with a kind of cover, forming what's called a corannulene cyclophane. The team hunted around and came up with several ideas for molecular bridges to cross the corannulene cap and so create useful cavities. They then used a standard synthetic chemistry approach to work out what simpler reagents they would need to build the bridge and how they could fix it to the top of the basin. The first bridge they built consisted of a benzene ring with two sulfur-containing branches acting as anchor points on the corannulene cap.

Since submitting their initial results to *JACS*, the team has also spanned the opening with a long-chain hydrocarbon bridge, turning the basin into a basket with a handle. They are now refining the chemistry to use one corannulene molecule to bridge another, going partway to the spherical buckyball shape in what Siegel calls a basketball molecule. Rabideau is intrigued by these particular spin-off molecules. "It will be interesting to discover what sort of things might be captured in the basket and how their properties might be modified," he says.

Such syntheses are of more than academic interest. For many potential applications for fullerenes, a bridged corannulene molecule would do just as well. And given the present awkwardness of dealing with fullerenes, this is an attractive proposition. Such applications could include sieving particular molecules from a mixed solution, if the target molecule were just the right size to slip into the corannulene cyclophane cavity—bigger molecules would not fit, while much smaller molecules would pass through untouched. A fluorescent marker attached to the corannulene might glow to signal the presence of a guest inside the cavity, turning the compound into a sensor. Corannulene cavities might also serve as catalysts by trapping large molecules, stretching their bonds, and making them more likely to undergo chemical reactions.

Siegel and his colleagues are also considering, once they have a double corannulene cyclophane, going the rest of the way and filling in the gaps to make a buckyball proper. As a way to produce buckyballs, such a chemical synthesis might be more efficient as well as more versatile than current arc synthesis. But fullerene pioneer Roger Taylor of Britain's Sussex University estimates that it will be at least 5 years before anyone succeeds in making the basic spherical buckyball in significant amounts by chemical routes.

For Siegel, the real goal is duplicating behavior, not form. "We are more interested in making compounds that mimic fullerene behavior but offer greater versatility in the structure and chemistry," he says. While others kick around buckyballs, Siegel hopes to get new applications in the basket.

–David Bradley

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