Careers in Biophysics

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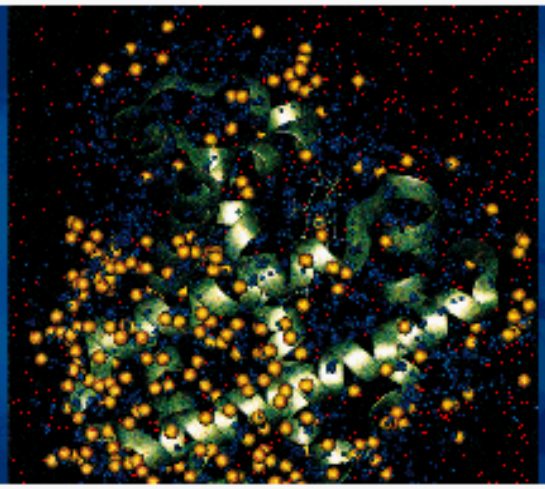

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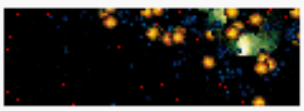

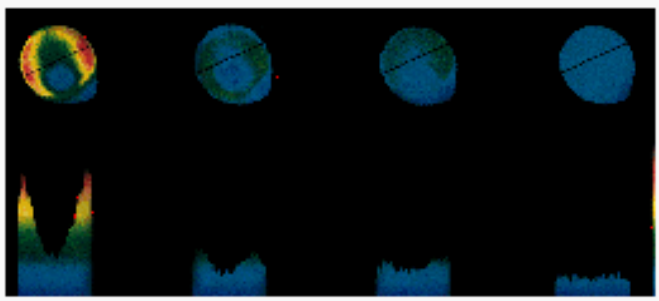

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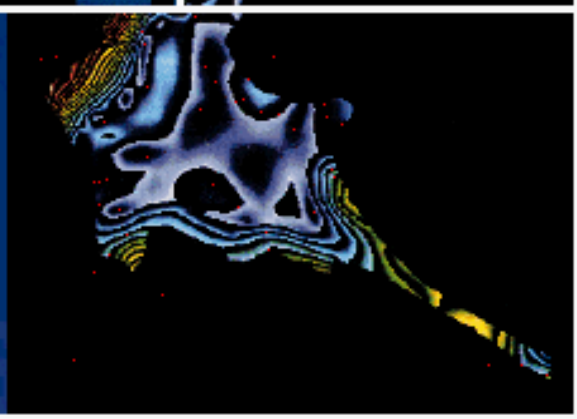

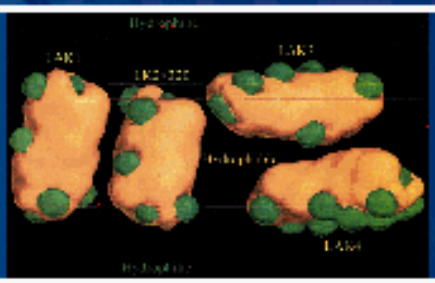

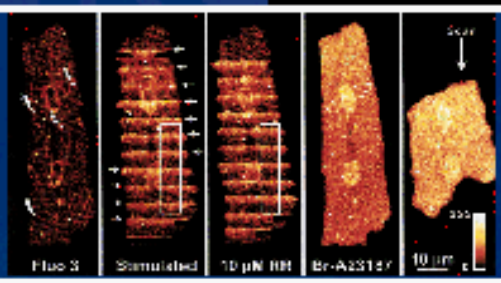

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Cover Captions

Clockwise from top

Photo 1: A view of how water is predicted to bind or hydrate the protein myoglobin (yellow dots). This prediction can be compared to the water sites found experimentally. Taken from *Residence Times of Water Molecules in the Hydration Sites of Myoglobin*, Pettitt et al, *Biophysical Journal*, December 2000, Volume 79, Number 6.

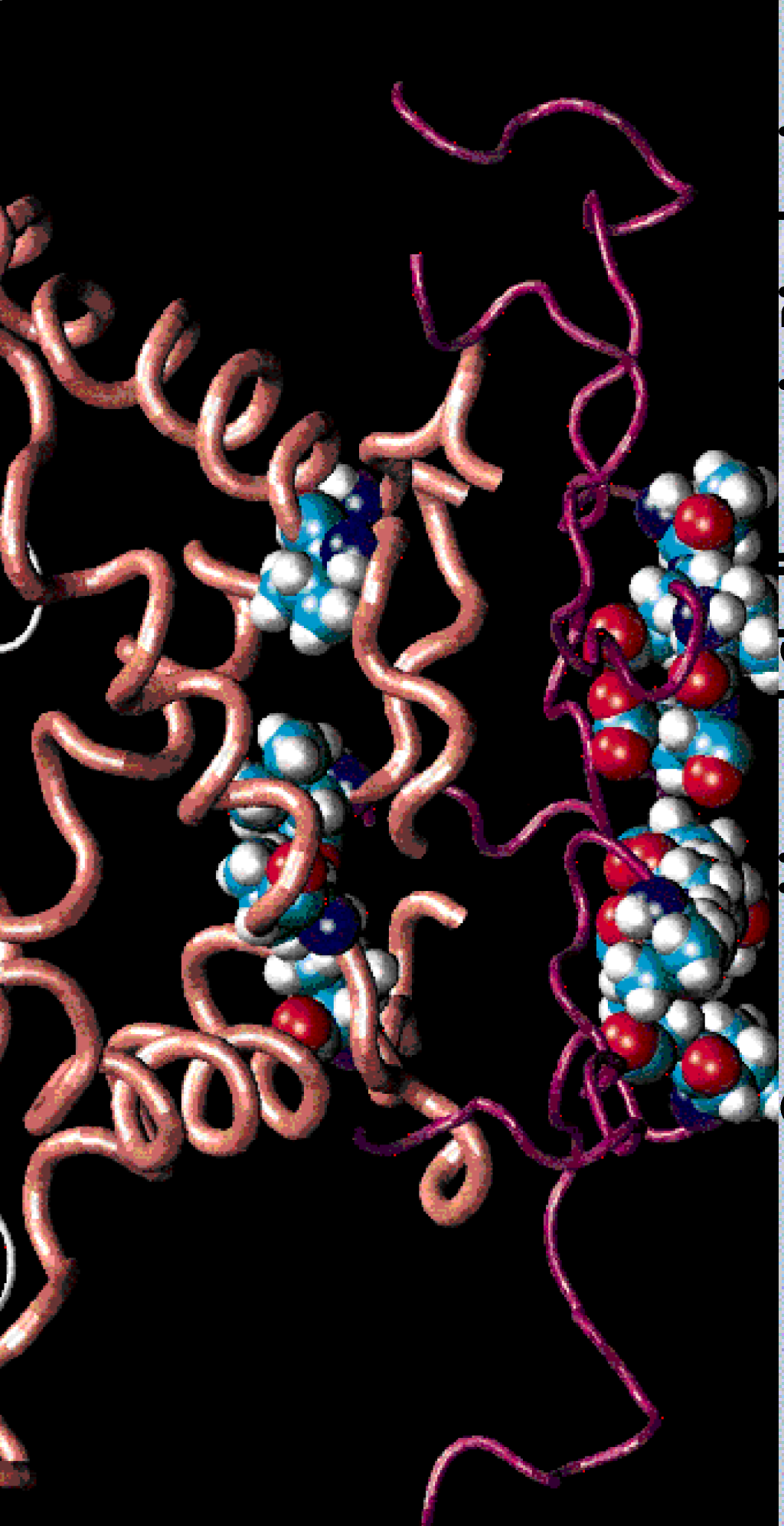
Photo 2: Time course of the development of Ca^{2+} gradients measured by pulsed laser imaging of a fluorescent calcium-sensitive dye in adrenal cells. Ca^{2+} gradients were induced by a 40-ms depolarization in patch-clamped adrenal chromaffin cells. Taken from *Development and Dissipation of Ca^{2+} Gradients in Adrenal Chromaffin Cells*, Marengo and Monck, *Biophysical Journal*, October 2000, Volume 79, Number 4.

Photo 3: Distribution of stress on a migrating N/H 3T3 cells caused by traction. Strong traction, shown in red, was constrained to a thin band along the leading edge of the cell. Taken from *Traction Force Microscopy of Migrating Normal and H-ras Transformed 3T3 Fibroblasts*, Wang et al, *Biophysical Journal*, April 2001, Volume 80, Number 4.

Photo 4: Helical orientations of lysine-containing model peptides in oriented lipid bilayers observed by molecular modeling and solid-state nuclear magnetic resonance spectroscopy. Taken from *The Topology of Lysine-Containing Amphipatic Peptides in Bilayers by Circular Dichroism, Solid-State NMR, and Molecular Modeling*, Bechinger et al, *Biophysical Journal*, November 2000, Volume 79, Number 5.

Photo 5: Rapid Ca^{2+} signals in heart cells brought about by electrical stimulation. In this experiment, the authors tried to inhibit the currents by the dye Ruthenium Red. Taken from *Mitochondrial Calcium Transients in Adult Rabbit Cardiac Myocytes: Inhibition by Ruthenium Red and Artifacts Caused by Lysosomal Loading of Ca^{2+}-Indicating Fluorophores*, Lemasters et al, *Biophysical Journal*, July 2000, Volume 79, Number 1.

This publication is a revision of the original Careers in Biophysics, published in 1990. The revision was conducted by the Society's Education Committee, in particular Suzanne Scarlata, Chair, Mark Milanick and Kathleen Howard.

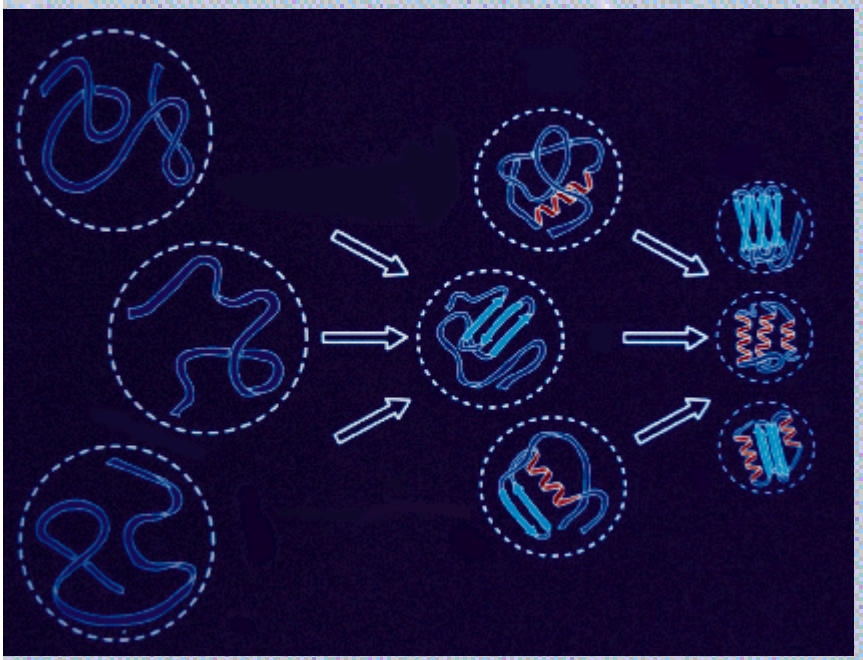


Opportunities and Challenges in Biophysics

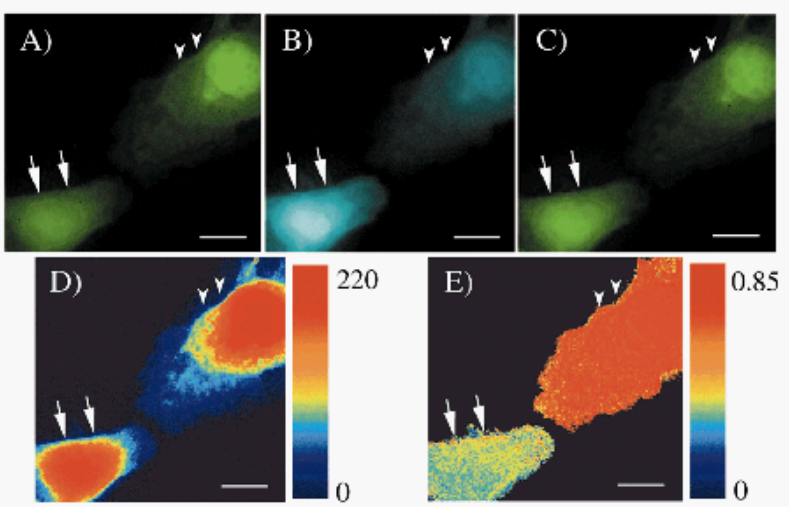
If you are curious about biological processes and enjoy puzzle solving, designing experiments or working with numbers and computers, there are many exciting opportunities for you in biophysics. Biophysicists use the methods of mathematics, physics, chemistry and biology to study how living organisms work. They investigate how the brain processes and stores information, the heart pumps blood, muscles contract, plants use light in photosynthesis, genes are switched on and off and many other questions. Other kinds of scientists, including physiologists, cell and molecular biologists, geneticists and biochemists, also work on these problems; however, biophysicists are especially interested in the physics and physical chemistry of biological processes and make far greater use of quantitative measurements and analysis. Biophysicists work in universities, industry, medical centers, research institutes and government. Women and minorities are actively being recruited. This booklet discusses some of these opportunities.

From Organs to Molecules

Biophysicists ask questions at many levels. At the highest level of organization, they study how organisms develop, see, hear, taste, feel and think. They also study how we breathe, how materials travel through our bodies, how our immune systems work, how muscles contract and how our bones support us. Other biophysicists look at biological processes on the scale of the single cell. They investigate how cells move, divide, and detect and respond to signals from the environment, and how materials travel into and through cells. Still other biophysicists study the structure and behavior of the biomolecules that make up cells. Very large molecules such as DNA and proteins are of particular interest. The ability of these molecules to perform complex biological tasks depends on their three-dimensional shapes and also their dynamic properties; therefore the relationship of structure to function is a central question.



Confinement of a polymer chain to a compact region is an important factor that contributes to helical and sheet substructures. In proteins, folding appears to be driven mainly by hydrophobic interactions. Reprinted with permission from *The Protein Folding Problem,* Chan and Dill, *Physics Today,* February 1993.



Measurement of an optical parameter that indicates when two fluorescent molecules are within approximately 50 Å in cells. The figure shows a cell expressing two fluorescent proteins at a ratio of 2:1 (arrowheads) and 4:1 cells. Taken from *Reliable and Global Measurement of Fluorescence Resonance Energy Transfer Using Fluorescence Microscopes*, Xia and Liu, *Biophysical Journal,* October 2001, Volume 81, Number 4.

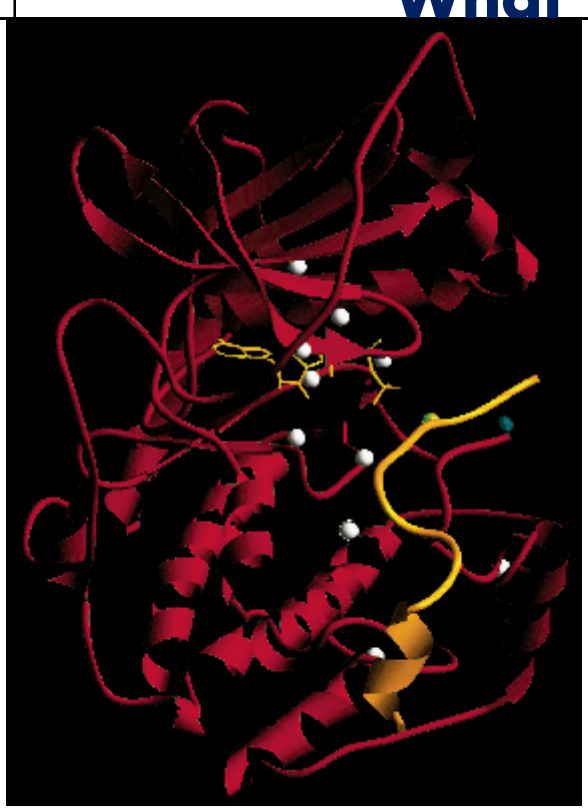
It is difficult to believe that only a few generations ago, the fundamental forces behind life were thought to be different from the forces that, for example, drive steam engines, cause lightning and create rock formations. Today we know that although life retains unique properties that cannot yet be fully explained, it can be understood as a complex of fascinating and intricate biological "machines." The properties of biological molecules and groups of molecules that convey their structure, dynamics, electrical properties and so forth, can be understood in terms of familiar physical and chemical laws. Such is the work of the biophysicist.

Biophysicists use methods and instruments designed by physicists and physical chemists. Most approaches can be used in many ways. For example, a technique called nuclear magnetic resonance spectroscopy (NMR) detects transitions between spin states of atomic nuclei in strong magnetic fields. This method can be used both to create images of individual molecules and to detect tumors in the body.

Computers are used extensively for data collection and analysis, as well as for modeling learning and memory, the flow of material through metabolic pathways, and motions of atoms within large molecules. In the future, as larger, faster computers become available, biophysicists will use them to analyze the mass of information about the chemical structure of human genes and chromosomes that the Human Genome Project will provide.

The discoveries of biophysicists have a broad impact on biotechnology and medicine. Biophysics gives us the tools to understand the molecular basis of diseases such as sickle cell anemia and AIDS. Knowledge of the way proteins and membranes work provides a basis for rational drug design. By understanding how proteins fold and accelerate chemical reactions, biotechnologists will be able to design proteins to carry out specific tasks, such as digesting crude oil and toxic wastes.

What Do Biophysicists Do?



The catalytic subunit of a protein kinase with an energy activator ATP and an inhibitor. With the human genome predicted to encode over 2000 of these types of proteins, this is perhaps the largest gene family. Opening and closing of the cleft is an essential functional feature of these proteins. Taken from *Biophysical Society 2000 Call for Papers*, Susan Taylor, National Lecturer.

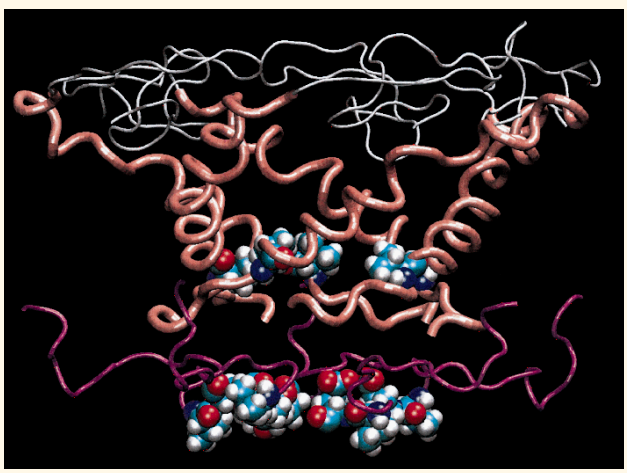
Because biophysics is developing so rapidly, it is often mistaken for a relatively new science. However, the origins of biophysics go back to the Greeks and Romans who first developed tentative hypotheses about the physical basis of consciousness and perception. The artist Leonardo da Vinci and others investigated the mechanics of animal motion during the Italian Renaissance. During the 18th and 19th centuries, Galvani discovered that electricity makes muscles contract, Thomas Young experimented with lenses, and Mayer and Helmholz investigated the relationships between work, heat and energy.

Mathematical biophysics developed rapidly in the early twentieth century when D'Arcy Wentworth Thompson analyzed the geometry of animal form and Haldane and others studied the chemical dynamics of cells. Walther Nernst's work with voltaic cells during the same period provided the basis for contemporary studies of membrane potentials and ion channels, which regulate everything from neural firing to muscle contraction.

The field of **molecular biophysics** arose at the close of World War II when a group of atomic physicists became interested in biological processes. Molecular biophysics has both **informational** and **structural** components. The informational approach emphasizes the transmission of genetic information within an organism and from generation to generation, while structural molecular biophysics focuses on the structures of biomolecules and biomolecular assemblies and the relationships of theses structures to biological function.

In the quarter century following World War II, new physical methods were developed that greatly accelerated the pace of discovery: **analytical ultracentrifugation** separates molecules in a centrifugal field on the basis of size; electrophoresis separates molecules according to charge; **spectroscopic methods** reveal details of structure by detecting interactions with **electromagnetic** radiation; sophisticated electronics enables detailed studies of membrane potentials and ion channels; **X-ray crystallography** makes it possible to view three-dimensional structures at atomic resolution. Indeed, this method provided the first clues that DNA is a double helix.

In recent years, many biophysicists have become interested in investigating the physical basis of biological function of specific molecules, cellular organelles or organs and in refining existing methods so as to be able to probe these systems in greater detail. Some currently exciting problems are discussed under "Questions Biophysicists Ask."



Computer calculations of the movements of a protein after applied surface tension simulation, for 113 picoseconds (10^{-10}s). Taken from *Structural Determinants of MscL Gating Studied by Molecular Dynamics Simulations*, Gullingsrud et al, *Biophysical Journal*, May 2001, Volume 80, Number 5.

A Little History

Meetings and Journals

Biophysicists from every setting meet annually at the national meeting of the Biophysical Society and may also participate in the national and international meetings of other scientific societies. Smaller meetings such as the Biophysical Discussions, Gordon Research Conferences and the UCLA-ICN meetings focus on particular areas. American biophysicists also participate in meetings in other countries, often coordinated by the International Union for Pure and Applied Biophysics (IUPAB).

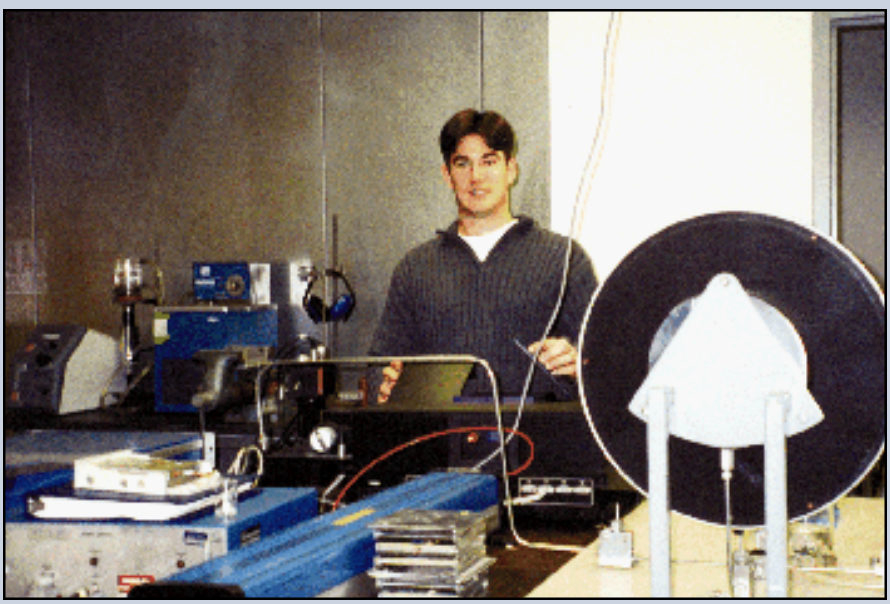
The Biophysical Journal is the official journal of the Biophysical Society. Biopolymers, Biophysical Chemistry, Proteins, Protein Engineering and several other journals also

A wide range of careers are open to biophysicists because of the breadth of their training. Depending upon your interests and abilities, you might work primarily in the laboratory, with computers, teach or become a science writer.

Many biophysicists become faculty or staff members at colleges, universities, medical or dental schools. There will be many openings for young faculty members in the next two decades. Faculty members at liberal arts colleges work primarily with undergraduate students and direct research programs that both generate new knowledge and provide experience for undergraduates. Faculty members at universities and medical and dental schools train graduate students and postdoctoral fellows to do research; they also teach undergraduates or medical or dental students. Their laboratories are generally supported by grants from federal agencies and private foundations.

Biophysicists whose primary interest is in research often work in government, private research institutes or industry. For example, biophysicists at the National Institutes of Health in Bethesda, Maryland, study the molecular and cellular basis of disease. Others work at National Laboratories in Brookhaven, New York, Argonne, Illinois, Los Alamos, New Mexico, or Oak Ridge, Tennessee; Naval Research Laboratories; Departments of Agriculture or Defense; the National Aeronautics and Space Administration or in private research institutes. Many new positions have been created in industry as a result of recent developments in molecular biophysics and molecular biology.

Regardless of the setting, biophysicists generally work in groups with people with different backgrounds, interests and abilities who collaborate to solve common problems. Everyone shares the adventure of embarking into unexplored territory and the thrill of discovery.



Paxton Provitera, a graduate student, working on apparatus to take optical measurements at a high hydrostatic pressure. These types of measurements can be used to study the dissassembly of viruses such as HIV. Photo courtesy of Suzanne Scarlata, SUNY Health Science Center, Stony Brook. Used with permission.

Where Do Biophysicists Work?

emphasize biophysics. Major papers frequently appear in prestigious but less specialized journals such as Biochemistry; Journals of Biological Chemistry, Cell Biology, General Physiology and Molecular Biology; Nature; Proceedings of the National Academies of Science (USA); Science; and the EMBO (European Molecular Biology Organization) Journal. Reviews in biophysics are published in the Annual Review of Biophysics and Biophysical Chemistry and Quarterly Review of Biophysics. Scientific American is a wonderful source of articles for the nonspecialist.

The First Steps

Most people who become biophysicists discover in high school that they are curious about natural phenomena, enjoy puzzles and problem solving and like designing and making things. If you have the opportunity, you might like to enter a science fair, assemble a piece of electronic equipment, experiment with computers or try your hand at photography. You should be sure to take courses in English, biology, physics, chemistry and mathematics. It may be possible to get laboratory experience in a high school or in a summer program at a local college or university; several programs are supported by the National Science Foundation and private foundations.

A postdoctoral fellow, Yuanjian Gao (top) looks at experimental results generated by graduate students Vijaya Narayanan (left) and Louisa Dowal (right). Photo courtesy of Suzanne Scarlata, SUNY Health Science Center, Stony Brook. Used with permission.

How Do I Become a Biophysicist?

College Years

Very few colleges or universities offer an undergraduate major in biophysics. Most students prepare by completing a major in physics, chemistry or mathematics with supplementary courses in biology. It is also possible to major in biology, biochemistry or molecular biology and take supplementary courses in chemistry, physics and mathematics; however, most students find that majoring in a physical science or mathematics is the best preparation for advanced work.

The ideal program would include the following:

Biology: Introductory biology, cell biology, molecular biology, genetics.

Physics: Mechanics, electricity and magnetism, optics, atomic and molecular physics.

Chemistry: General chemistry, organic chemistry, physical chemistry.

Mathematics: Calculus, differential equations, linear algebra, numerical analysis and statistics, computer programming.

For a well-rounded education, it is important also to take courses in the humanities and social sciences and to participate in extracurricular activities. Because science transcends national boundaries, courses in foreign languages often prove useful and are sometimes required by graduate schools.

Hands-on research experience is essential so that you begin to learn how scientists tackle real problems. Science courses often have accompanying laboratory sessions; however, many students get their first real taste of research from a summer job in a laboratory. You can find out about these opportunities by contacting the chairperson or faculty members at your college, the National Science Foundation or the Howard Hughes Medical Institute.

By your junior year, you will want to consider whether to proceed immediately to advanced training. If you want to become involved in research, but do not want to continue on to graduate or medical school, you will probably want to explore openings for technicians. Other possibilities include teaching at the elementary or secondary school level or working for the government or media.

Graduate Study

If you want to become an independent scientist, you must earn a PhD or MD/PhD degree. Universities and medical schools offer PhD degrees; only medical schools offer combined MD/PhD degrees. Holders of MD/PhD degrees are able to practice medicine as well as do research. Earning a PhD usually requires four to six years; the combined degree takes somewhat longer.

During the first couple of years of graduate school you will take advanced courses and attend seminars to broaden and deepen your scientific background. You also will work in two or three laboratories to learn specialized procedures and decide where to do your thesis research, and take a set of examinations that qualifies you to do PhD research. However, your major goal in graduate school is to complete a solid piece of research that merits publication in a professional journal.

Most graduate students hold teaching or research assistantships that provide stipends in the range of $15,000-$20,000 per year. They often are not required to pay tuition for courses. Interest-deferred loans also are available.

How do you go about choosing a graduate school? Although a number of universities and medical schools have departments of biophysics, biophysics and biochemistry or biophysics and physiology, biophysics programs often are interdisciplinary and sponsored by several different departments, or part of a department of biochemistry, cell biology, chemistry, neurobiology, pharmacology, physics or physiology. Information about graduate programs in biophysics can be obtained from the office of the Biophysical Society. Peterson's Guide to Graduate Education or Web sites such as www.gradschools.com are other useful sources.

Once you have identified several graduate schools that interest you, write to them for brochures. Bear in mind that most graduate applications are due six to nine months in advance of entrance. You should also plan to take the Graduate Record Examinations; schedules can be obtained from the Educational Testing Service (Princeton, New Jersey).

It is important to get advice from professional biophysicists since graduate programs differ enormously in philosophy, size, research opportunities, overall quality and reputation. You can get the names of biophysicists who act as advisors by contacting the office of the Biophysical Society. You also should visit schools to evaluate them firsthand.

One of the major considerations in choosing a graduate school is your research advisor. The faculty member whom you select to direct your research will play several roles: advisor, role model and, with luck, friend. The program you enter should include several faculty members who share your research interests, maintain active research programs and are people with whom you would enjoy interacting.

Postdoctoral Research

After earning your PhD, you will probably want to spend a few years in another laboratory to expand your range before accepting a permanent position. Postdoctoral work provides an opportunity to pursue research with the wisdom gained from graduate school and without the administrative responsibilities of more senior scientists. Some postdoctoral fellows are supported from research grants to the host laboratory. Others are awarded fellowships from the National Institutes of Health or private foundations.

International Opportunities

Postdoctoral years also provide the opportunity to gain research experience at the international level and establish fruitful and unique collaborations with biophysicists of other countries. At the same time, fellows have the opportunity to know other cultures and ways of living. This is important as science is an international endeavor. Support can be obtained from the National Science Foundation that has an International Research Fellowship Program (IRFP) (http://www.nsf.gov/cgi-bin/getpub?nsf01135) or from the host laboratory.

Louisa Dowal, a graduate student, prepares a virus containing the DNA of a cell signaling protein linked to a naturally fluorescent protein. This will allow her to see where the protein is in a neural cell and how its localization changes when the cell is stimulated with a neurotransmitter using fluorescence microscopy. Photo courtesy of Suzanne Scarlata, SUNY Health Science Center, Stony Brook. Used with permission.

Questions Biophysicists Ask

The next decade promises to be particularly interesting for biophysicists. Dramatic improvements in determining structures and obtaining large quantities of materials through recombinant DNA technology and sophisticated computers and electronics have made it possible to study the inner workings of biological systems with unprecedented precision. These brief summaries are not comprehensive; rather, they are intended to convey some of the flavor of some areas of current research. For more information, consult the references listed in each section.

Structural elements in *C. elegans* (a type of small worm) embryos taken within the uterus of a live mother. Eight embryos are shown at low magnification. Stages seen include pronuclear rotation in the zygote (far left), mitosis in the first cell division (far right), interphase in the two-cell stage (second from left), as well as various stages of early embryonic cleavage. Taken from *Three-Dimensional High-Resolution Second-Harmonic Generation Imaging of Endogenous Structural Proteins in Biological Tissues*, Campagnola et al, *Biophysical Journal*, January 2002, Volume 82, Number 1.

How Do Proteins Fold?

One of the major questions in biophysics is the question how three-dimensional structure determines biological function. Why do molecules and parts of molecules assume the shapes they do? How do they fold into these shapes, and how do they change their structure under changing conditions? The shapes molecules take depend on the physical and chemical forces acting upon them and within them. With methods such as X-ray crystallography, nuclear magnetic resonance spectroscopy and scanning probe microscopy, biophysicists can determine the three-dimensional structures of molecules.

Proteins, in particular, function biologically in ways that depend precisely on the three-dimensional shapes assumed by the sequence of amino acids that make them up. A protein folded in one way may create a binding site for a small molecule. When the small molecule binds to the protein, it sets up a complex set of reactions. If the protein does not fold in this particular way, the binding site will not be there and the reaction will not occur. But what precisely are the physical forces involved in this folding? How can we predict whether a certain protein will fold in one way rather than a million other possible alternatives?

X-ray crystallography has clearly revealed certain characteristic folding patterns; for example pieces of the chain often organize themselves as helices or pleated sheets. Biophysicists have only begun to discover the hierarchy of rules that determines folding patterns. For example, certain amino acids have an affinity for water while others have an aversion. The former are said to by hydrophilic, the latter hydrophobic. Proteins fold with hydrophilic amino acids outside, in contact with water, and hydrophobic amino acids inside, shielded from water. In addition, proteins tend to fold compactly, in ways that leave few holes in the structure. Moreover, the folding must produce a shape that is complementary to the shapes of other molecules with which they interact.

Various forces, including hydrogen bonds and van der Waals contacts, maintain the shapes of proteins. For example, protein chains may form coils that are held together by hydrogen bonds. However, deciphering the code that determines the three-dimensional structures of proteins is a daunting task, especially because the shapes are very complex and have yet to yield a simple set of rules.

Two recent developments have made it possible to begin to crack this "second genetic code." First, recombinant DNA technology can produce substantial quantities of almost any protein. This allows biophysicists to search for patterns of folding under different circumstances and thus to begin to deduce the underlying "rules." Second, this technology and powerful methods of synthesizing segments of proteins make it possible to cut up the chains, studying single segments at a time. Biophysicists can ask: What happens if one amino acid is removed and replaced by another? Adding and subtracting amino acids makes it possible to ask which pieces are essential, which are redundant, which enhance or eliminate certain kinds of behavior.

Such knowledge has obvious and immediate applications in the synthesis of new kinds of medicines and products. Ideally, an understanding of rules for protein folding would make it possible, for example, to design a protein that would fold into the precise shape required to recognize an abnormal protein on the surface of a cancer cell and attack and destroy the cell.

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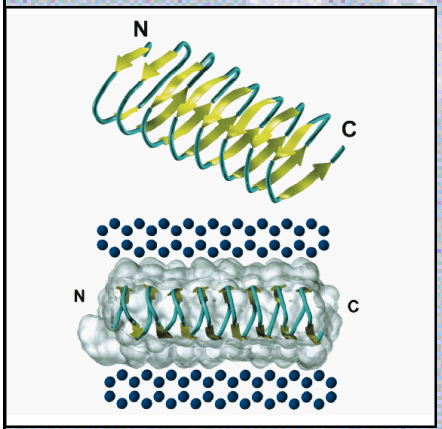
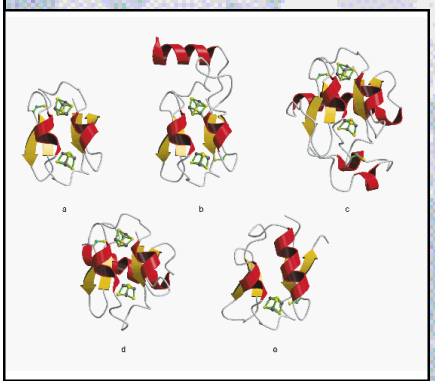


A model of the perennial ryegrass antifreeze protein showing different key structural features. The protein is postulated to align between two prism faces of ice (blue spheres), thereby stopping ice boundary migration and essentially acting as an interfacial "glue" to prevent ice recrystallization. Taken from *A Theoretical Model of a Plant Antifreeze Protein from Lolium perenne,* Kuiper et al, *Biophysical Journal,* December 2001, Volume 81, Number 6.



Ribbon representation of the structures of [4Fe-4S]-type ferredoxins from Peptostreptococcus asaccharolyticus (a), Chromatium vinosum (b), Azotobacter vinelandii (c), Bacillus schlegelii (d), and Desulfovibrio africanus (e). These ferredoxins all have sulfer atoms near one of their [4Fe-4S]-type clusters, which are shown in ball-and-stick representation with sulfurs (yellow), irons (magenta), and carbons (cyan). These sulfers lead to a decrease in the reduction potential of the nearest cluster because of differences in the electrostatic environment, which changes the functional properties of the protein. Taken from *Sequence Determination of Reduction Potentials by Cysteinyl Hydrogen Bonds and Peptide Dipoles in [4Fe-4S] Ferredoxins,* Beck et al, *Biophysical Journal,* August 2001, Volume 81, Number 2.

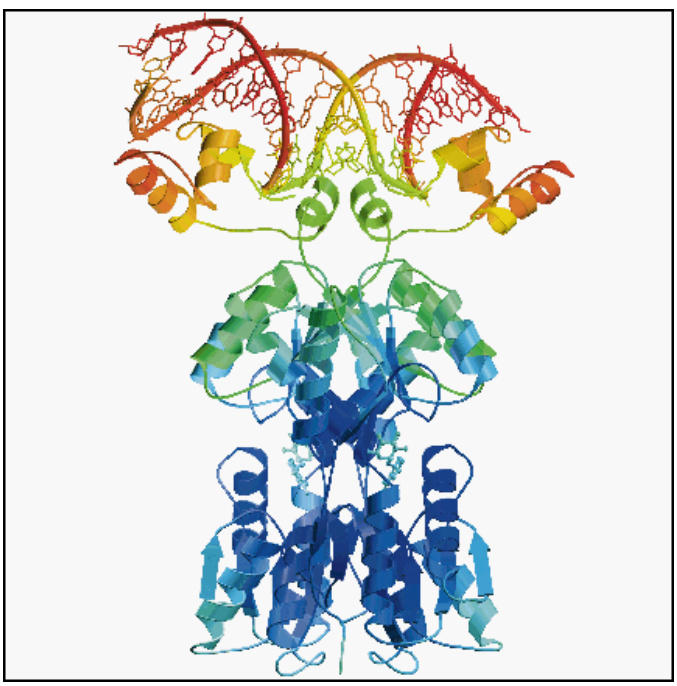
How are Genes Turned On and Off?

The discovery of the double helical structure of the DNA molecule was a breakthrough for many branches of biology, because it explained how the structure of the molecule allows it to function as a template for copying genetic information. However, it was not clear how DNA works in three dimensions – what sorts of three-dimensional structures are superimposed on the double helix and how these influence the interactions of DNA with other molecules in its environment.

Binding of proteins to DNA determines when and how genes are turned on and off and in turn regulates both normal and abnormal development. The proteins in effect read the code and determine which segments will be manipulated, duplicated or regulated. By turning genes on and off, the proteins control which parts of the genetic information will be expressed, where, how and when. Gene expression in turn determines everything from the development of diseases such as cancer and multiple sclerosis, to puberty, aging and even some aspects of behavior.

In recent years, recombinant DNA technology has made it possible to obtain large amounts of DNA and the proteins that interact with it. Biophysical methods are being used to elucidate the rules that determine where and how proteins bind to DNA and the nature of the protein complexes that allow DNA to be transcribed to RNA and then made into a protein.

Still, techniques such as crystallography yield essentially static pictures. To obtain a dynamic picture of how DNA interacts with proteins, biophysicists have adapted a technique from molecular biology. Called "footprinting" because of the telltale patterns generated, the experiment begins with a solution of DNA, protein and a chemical or enzyme that breaks the DNA chain into pieces. Wherever the protein binds to the DNA, it protects the DNA from being degraded. As the concentration of the protein increases, more and more binds to specific sites, preventing cleavage. By monitoring the patterns of cleavage, the experimenter can see exactly how much protein binds to various sites on the DNA, and in turn begin to understand how interactions between DNA and proteins regulate gene expression.



Structure of a DNA binding protein bound to its target DNA. This binding leads to a decrease in transcription. Reprinted with permission from *A Closer View of the Conformation of Lac Repressor Bound to Operator*, Bell and Lewis, *Nature Structural Biology*, Volume 7, Number 3, March 2000.

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How Do Biomolecules Assemble and Interact to Perform Biological Functions?

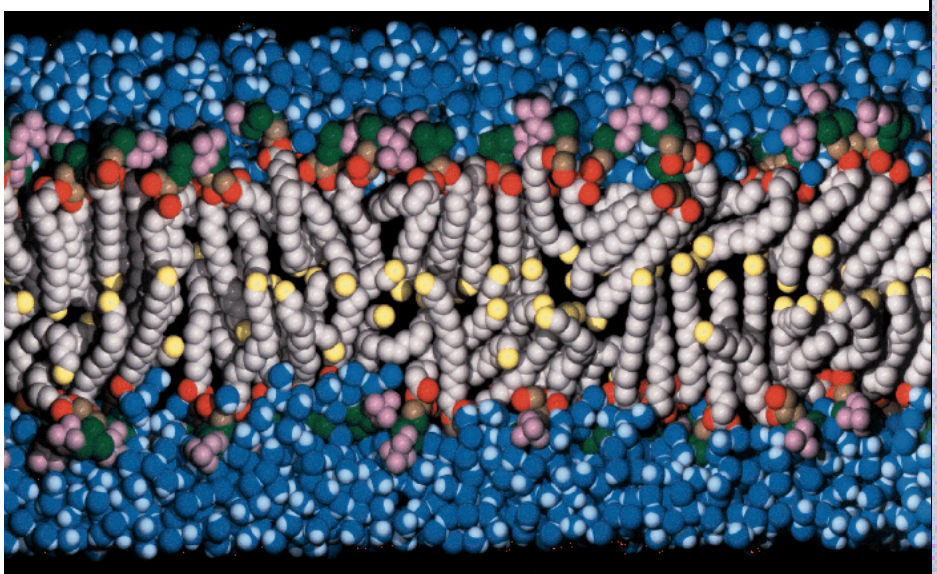
Understanding how the structure of macromolecules determines their behavior is only the beginning; most biological events involve interactions among assemblies of macromolecules working together. A cell, for example, can be thought of as a complex machine composed of simpler machines. These large assemblies perform all of the familiar functions of biological systems, including replication of genes into proteins, binding of antigens by antibodies, muscle contraction, transmission of nerve impulses and so forth.

Methods such as X-ray crystallography cannot easily be scaled up to view complex structures, each containing hundreds of thousands of atoms, and so other methods such as electron microscopy are required. In the series of images to the right, one can see clearly how the individual protein molecules that make up the core of a virus can be visualized by X-ray crystallography, while electron microscopy allows one to view the whole virus. The biophysist can then ask: How can the virus enter its target cell? How does it replicate in the cell? How do new virus particles leave the cell?

In order to understand how these biological "machines" work, one must understand the forces that drive them. The driving force for their self assembly and operation is free energy. Each possible structure of an assembly of macromolecules has a different free energy, which, in turn, determines how likely the assembly is to assume that structure. These different energy states operate at different parts of the biological cycle; they both drive the cycle and are driven by it.

Free energy has two components: enthalpy and entropy. Enthalpies depend upon attractions and repulsions between atoms, whereas entropy is a measure of disorder or degeneracy. Although entropy naturally tends to increase, it can drive the formation of biomolecular assemblies when the formation of structure in the assembly is coupled to a decrease in the order of the environment. Both the enthalpy and entropy of biological systems generally depend heavily on noncovalent bonds such as hydrogen bonds and van der Waals contacts.

When the driving forces have been elucidated, models can be developed that predict how the assembly will behave under different conditions. This approach has been used to show how binding of the cI repressor to DNA helps control the life cycle of lambda bacteriophage.



A computer simulation of the movement of lipid molecules in membranes. Atoms and atom groups are colored as follows: yellow, chain terminal methyl; gray, chain methylene; red, carbonyl and ester oxygen; brown, glycerol carbon; green, phosphate; pink, choline; dark blue, water oxygen; and light blue, water hydrogen. Reprinted with permission from *Computer Simulation of a DPPC Phospholipid Bilayer: Structural Changes as a Function of Molecular Surface Area,* Feller et al, *Langmuir,* Volume 13, Number 24, 1997. American Chemical Society.

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What is the Molecular Structure of Cell Membranes?

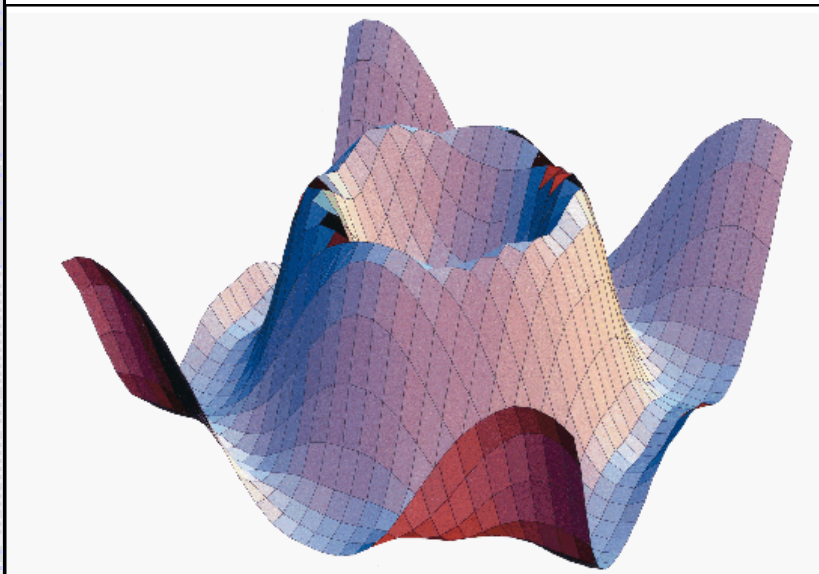
The membranes of the cell carry out a diverse multiplicity of functions that are essential for life. Membranes compartmentalize cells and form barriers between different environments. They also move molecules from one part of the cell to another by recognizing elements of molecular structure that indicate where in the cell the molecule belongs. In addition, membranes play a key role in energy transformations, taking light or chemical energy and converting it into other forms that can be used by the cell.

The lipid molecules that make up most of membranes have an affinity for both oil and water; that is, they have both hydrophobic and hydrophilic groups. They naturally tend to line up in a bilayer with their hydrophilic groups on the outside and their hydrophobic groups on the inside. Embedded in this barrier are complex proteins that serve as molecular ports, allowing certain kinds of molecules to cross the barrier, but not others. Both lipids and proteins are able to move around within the bilayer. This combination of hydrophobic and hydrophilic, structured and unstructured, raises a variety of questions about the relationship between the structure of membranes and their biological function.

For example, a specific membrane protein is responsible for transporting sodium and potassium ions across the membrane of many types of cells. This process is the chemical basis for all nerve impulses; only if the ion channel is gated (able to open and close) can impulses sweep from one end of the nerve to the other, transmitting signals.

How does such a membrane protein, synthesized in the aqueous cytoplasm, get into the lipid bilayer? Some membrane proteins appear to be synthesized "on-site" and inserted into the membrane as they are made. Others are produced elsewhere, then carried to the membrane by "chaperone" proteins. A third possibility is that proteins change structure en route, perhaps essentially turning "inside out" so that hydrophobic and hydrophilic surfaces reverse.

Even the lipid bilayer is not fully understood. The lipids in the two halves of the bilayer often differ in composition. How does this asymmetry arise and why does it exist? Does it play a role in transmitting signals across the membrane? To help answer these questions, biophysicists study isolated lipids and proteins; that is, they probe the structure and function of individual components of the membrane and their interactions in order to better understand the functioning of the whole.



A three-dimensional reconstruction showing a different structure that lipids can form besides membrane bilayers. Taken from *X-Ray Diffraction Structures of Some Phosphatidylethanolamine Lamellar and Inverted Hexagonal Phases,* Harper et al, *Biophysical Journal,* November 2001, Volume 81, Number 5.

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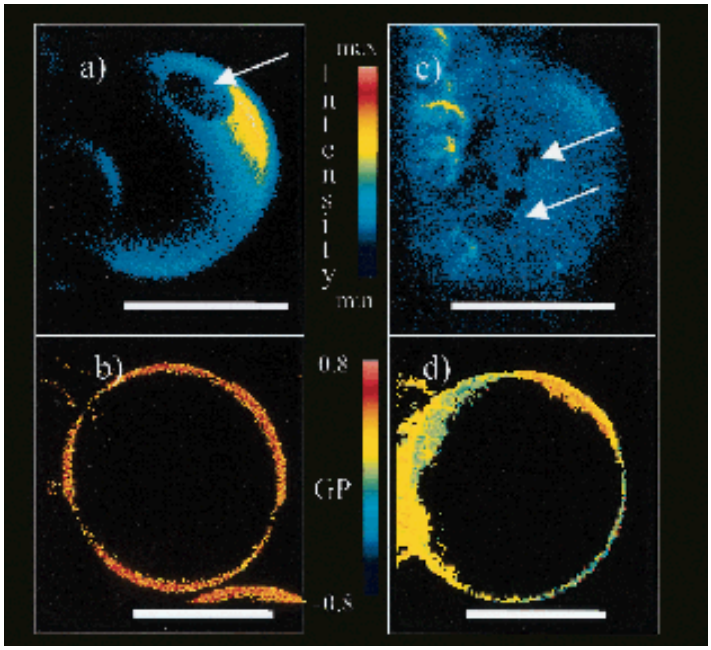
How Do Membrane Proteins Transduce Energy?



Fluorescence images of polar sections (a + c) equatorial sections (b and d) of membrane bilayers. Images demonstrate coexistence of solid-like phases in bilayers when cholesterol is present (a and b) at higher temperatures or when cholesterol is extracted (c and d) and the temperature is lowered. Scale bars, 50 μm. Taken from *Lipid Rafts Reconstituted in Model Membranes*, Dietrich et al, *Biophysical Journal*, March 2001, Volume 80, Number 3.

The interconversion of different forms of energy is essential for life. This interconversion usually involves membranes. For example, energy from the sun is transformed by plants into chemical energy that is stored as carbohydrates, which in turn are consumed by animals and transformed into the energy needed for development, motion and thought. The same energy from the sun when detected by the eye is transformed into chemical and then electrical signals which we perceive as vision.

The ability of membranes to change energy from one form to another depends on their unique structure. Proteins designed to transport molecules are aligned in the membrane so that they can generate concentration gradients; if the molecules are ions, then a transmembrane electrical gradient is also generated. The membrane bilayer maintains ionic gradients much as a flashlight battery separates electrical charges. The transmembrane electrical potential difference, referred to as a membrane potential, can be used to perform various tasks. Energy released by discharging an ionic or chemical gradient can be used to synthesize new compounds or to drive cellular processes. At the same time, the gradient is continually regenerated through respiration or the input of light or chemical energy, just as a battery can be continually recharged. Often the energy released when a gradient is broken down is stored as chemical energy by the high energy molecule, ATP, while the generation of an ionic gradient is driven by the breakdown of ATP.

The structures of energy-transducing membrane proteins are being determined using magnetic resonance techniques as well as X-ray crystallography and electron microscopy. These molecular structures and the ability to alter the proteins in specific ways (genetic engineering) have opened up the possibility of designing functional experiments to determine the mechanism of energy transduction at the molecular level. Research in energy transduction has applications that range from an understanding of mitochondrial myopathic disease to increased photosynthetic productivity.

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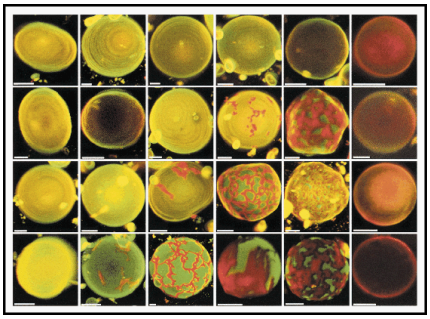
How Do Animals and Plants Respond to Light and How Do Animals Respond to Sound and Smell?

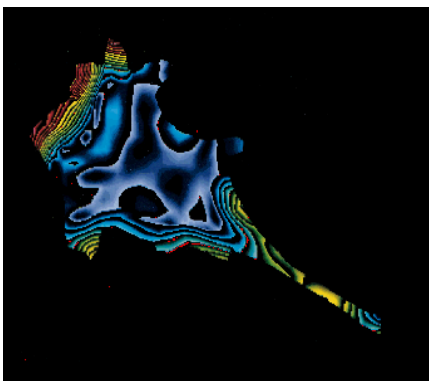
Proteins that transform energy in vision and photosynthesis are among the best understood of energy-transducing membrane proteins. The proteins that transform energy for hearing and smell are being actively investigated.

The first step in vision is the absorption of a quantum of light by the membrane protein rhodopsin. Within picoseconds (10 – 12 seconds), the photon triggers a series of transformations in the shape of rhodopsin that allows it to interact with a membrane-bound signalling protein, the G-protein. This interaction initiates an "enzyme cascade" that alters the permeability of the cell membrane by modulating the activity of ion channels. In turn the membrane potential changes, giving rise to the electrical signal detected by the brain. This mechanism allows for tremendous amplification of the light signal so that our visual system is sensitive enough to detect a single photon. Understanding the mechanism of this amplification will help us understand not only vision, but also hormone systems that use G-proteins in transmembrane signalling.

In contrast to vision, absorption of light by the photosynthetic systems of plants results in the transfer of electrons from one chlorophyll molecule to a chromophore-protein complex known as the reaction center. The reaction center is a complex protein that spans the membrane of the chloroplast and holds in place the reactive groups needed for the separation of charges, the first step in photosynthesis. Electrons are transferred from one group to another in a "bucket brigade." The crystal structure of the reaction center was determined in 1981. This was the first structure of a membrane protein determined at atomic resolution and it has had a profound influence on our understanding of photosynthesis. Dozens of laboratories around the world are now studying the mechanism of electron transfer in reaction centers using optical methods spanning timescales from femto (10 – 15) seconds to hours in combination with genetic manipulation of the protein itself.



Confocal fluorescence microscopy images of bilayers containing species that form solid-like patches in membranes. Cholesterol concentration varies along the vertical axis from 0 (bottom) to 16.5 mol%. Scale bars, 5 μm. Taken from *Ternary Phase Diagram of Dipalmitoyl-PC/Dilauroyl-PC/Cholesterol: Nanoscopic Domain Formation Driven by Cholesterol*, Feigenson and Buboltz, *Biophysical Journal*, June 2001, Volume 80, Number 6.



Distribution of stress on migrating NIH 3T3 cells caused by traction. Strong traction, shown in red, was constrained to a thin band along the leading edge of the cell. Taken from *Traction Force Microscopy of Migrating Normal and H-ras Transformed 3T3 Fibroblasts*, Wang et al, *Biophysical Journal*, April 2001, Volume 80, Number 4.

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How Do Cells Move?

The ability to move is one of the fundamental properties of all living cells. When the first spacecraft landed on Mars, one of the experiments attempted to detect the presence of life involved very sensitive instruments designed to determine whether or not anything moved.

The biological motion most familiar to us is generated by the skeletal muscles that move our limbs. Muscle proteins are not only found in animals; similar proteins have been isolated from other kinds of cells, including amoeba and plant cells. This discovery suggests a universal mechanism for motion — for plants and animals, cells and organisms. Indeed, the similarity of these proteins indicates that they evolved more than a billion years ago, before plant and animal cells diverged from each other in the evolutionary tree.

The force of muscle contraction is generated by two proteins called actin and myosin, which convert the chemical energy of ATP into the mechanical energy of force and motion. In non-muscle cells these proteins transport materials within the cell and help cells to move. Other sets of proteins in these cells function as motors.

In muscle tissue, myosin and actin form thick and thin filaments that are lined up and can be studied by tools such as X-ray diffraction, electron microscopy and fluorescence microscopy. These studies have told us much about how muscles work. When the muscle cell contracts, the thick and thin filaments slide past one another. A portion of the myosin molecule that reaches across and pushes the actin past it powers this sliding movement. Current studies focus on the exact molecular mechanism by which the energy of ATP is converted into the mechanical energy of muscle work.

Electrical signalling at the junction between nerve and muscle cells triggers the muscle fiber to contract. This signal is mediated by an increase in the concentration of calcium ions within the muscle cell. The sarcoplasmic reticulum, a membrane system within the muscle cell, contains the molecular machinery for calcium uptake, storage and release, and thereby regulates muscle relaxation and contraction. A transport protein in the membrane of the sarcoplasmic reticulum uses the energy released by the breakdown of ATP to concentrate calcium in the sarcoplasmic reticulum, where it is sequestered by a calcium binding protein. The electrical impulse from the nerve cell causes calcium to pass through a calcium release channel.

In the long term, it will be possible to manipulate the contractility of a number of muscles in our body: the cardiac muscles that pump blood, the smooth muscles of our arteries that control blood pressure and the smooth muscles of our gut that play a role in digestion. A number of drugs already in use treat high blood pressure by regulating the contractility of the smooth muscles of arteries.

When astronauts spend extended periods of time in space, their muscles waste away because they no longer have to fight against the forces of gravity. Thus, the first mission of humans to Mars will require a more detailed picture of how muscles work. To learn how to alleviate muscle atrophy in space we must first understand how muscle growth and regeneration are linked to muscle use and muscle fatigue.

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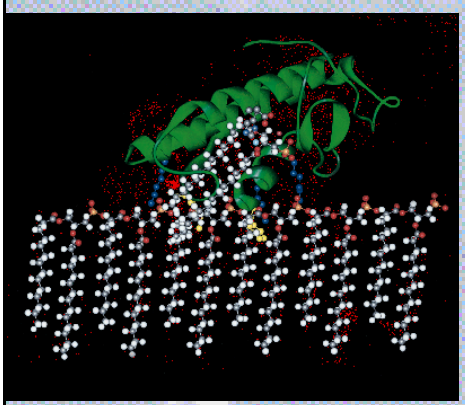

A phospholipid-digesting enzyme (green ribbon) bound to the surface of a phospholipid membrane. Originally taken from Han et al, 1997. J. Biol. Chem. 272:3573 and Pascher et al, 1987. and Biochim. Biophys. Acta 896:77. Taken from *Toward Understanding Interfacial Activation of Secretory Phospholipase A_2 (PLA_2): Membrane Surface Properties and Membrane-Induced Structural Changes in the Enzyme Contribute Synergistically to PLA_2 Activation,* Tatulian et al, *Biophysical Journal,* February 2001, Volume 80, Number 2.

How Does the Nervous System Work?

In one of the first great breakthroughs of biophysics in the 1940s, electrical circuits developed for radar were used to show that electrical signalling neurons result from the flow of sodium and potassium ions across the nerve membrane. These flows are "gated": that is, neuronal membranes are able to switch ionic flows on and off in milliseconds, by opening and closing ion channels. Much of the current research in neurobiology at the molecular level is aimed at understanding precisely how ion channel proteins work. Low-noise electrical instrumentation has made it possible to observe directly ionic currents mediated by individual channel molecules: that is, to look at a single molecule as it opens and closes the "gate."

Single-channel recording methods are now being combined with the surgical precision of molecular genetics to create ion channels with specifically designed mutations. Biophysicists can take a single protein, deliberately change its structure by substituting a single amino acid in the chain, and observe how its function is altered. These specifically designed mutant channels provide powerful opportunities to ask how channel proteins so cleanly distinguish among different ions, with sodium channels allowing only sodium ions to pass through, calcium channels only calcium ions, and so forth.

At the cellular level, a variety of quantitative techniques are used to understand how the cell's entire ensemble of ion channels acts to give rise to the electrical outputs that control what we think and how we move. For example, in response to hormones, neurons can "shape" their electrical signals by altering the structure of the channels, thus altering the flow of ions. Presently, much effort is going into discovering the pathways by which ion channels are "modulated" by changes in cellular environment.

Further up the neurobiological hierarchy, questions about how neurons talk to each other may be posed by observing electrical crosstalk among individual nerve cells in small systems of neurons. Invertebrate ganglia which contain 20–50 neurons often are used to understand how neurons work together to yield a coherent organ-directed output, for example, the coordinated contraction of several sets of muscles.

The subject of high-level neuronal integration has recently exploded as a field requiring mathematically sophisticated biophysicists. This purely theoretical area models the interactions of very large numbers of synapses, such as those in vertebrate ganglia and the brain. We now know that these kinds of models can show phenomena analogous to learning and memory. Understanding the rules governing these kinds of neural networks is one of the challenges of the future.

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