method of using two FETs to inject the current "is a beautiful idea, and it works!" There are also losses associated with carrier-induced absorption, but the problem can be avoided with proper choice of material to get the material to absorb at a different wavelength than that at which it emits. It remains to be seen whether the Bell Labs approach can be successfully generalized to other materials.

Part of the proof that the Bell Labs group had indeed formed a laser was the observed narrowing of the emission line as the current density increased—that is, as the laser was pumped harder and harder. As shown in the bottom figure on page 18 for two scales of energy, at low density the emission spectrum has several peaks. But as the gate voltages are raised to give higher current densities, the line continues to narrow. The first sign of optical gain sets in at current densities of about 30 A/cm^2, but the transition to lasing doesn't occur until about 500 A/cm^2. Above that threshold, the emission line is centered at about 580 nm. The laser operates in a continuous-wave mode up to 200 K and in a pulsed mode up to room temperature.

Vardeny noted two particularly nice features of the Bell Labs laser. One is the balance that is maintained between the current of electrons and holes. In present-generation light-emitting diodes, he points out, the holes outnumber the electrons and the holes that are left behind can serve as nonradiative combination centers, reducing luminescence. The balance in the tetracene laser is of course made possible only because the high-purity crystals have few traps for either electrons or holes. In most organic conductors, there are many more traps for electrons than holes, so that holes become the dominant carriers. Another special feature, Vardeny says, is the separation of controls: In the FET design, one can increase the current (through the gate electrode) without having to increase the voltage across the device.

Batlogg admits that the new laser is only a demonstration and is far from being optimized. "Most of the work is ahead of us," he claims. He and his colleagues believe they can reduce the threshold current by two orders of magnitude, for example. Among the obvious steps to further improve the laser are to get a proper feedback mechanism rather than the simple cleaved mirrors they now use and to introduce a low-loss waveguide surrounding the active region. Dodabalapur says they are working on resonator designs, including one based on photonic crystals. Such photonic crystals, particularly two-dimensional ones, will enable the laser light to be coupled out in technologically useful directions.

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Solid-State NMR Reveals Key Structural Features of Membrane Transport Proteins

With carefully chosen pulse sequences, practitioners of solid-state NMR are closing in on a much-sought prize in structural biology: the ability to unravel the molecular structure of membrane transport proteins.

Only small neutral molecules can pass unaided through cell membranes. Other kinds of particles—ions that mediate neural signaling, sugars that provide energy, amino acids that form proteins, and so on—enter and leave cells through specialized molecular portals known collectively as membrane transport proteins. To do their vital jobs, these proteins must recognize and grant passage, when required, to only one kind of molecule. And if getting that molecule into or out of a cell involves pushing against an electrical or concentration gradient, the transport protein has to marshal the necessary energy.

Membrane transport proteins can pull off these feats of molecular processing thanks to their intricate structures. And only by knowing these structures can biophysicists begin to discover how membrane transport proteins work.

Most proteins are found in the aqueous interiors of cells and cellular compartments, but membrane transport proteins inhabit a quite different environment: the flexible double layer of lipid molecules that constitutes the cell membrane. For a membrane transport protein to remain attached to the membrane, the protein's outer surface must match the electrical nonpolarity of the membrane's interior. This property renders the protein insoluble in water, hard to purify, and very difficult to crystallize.

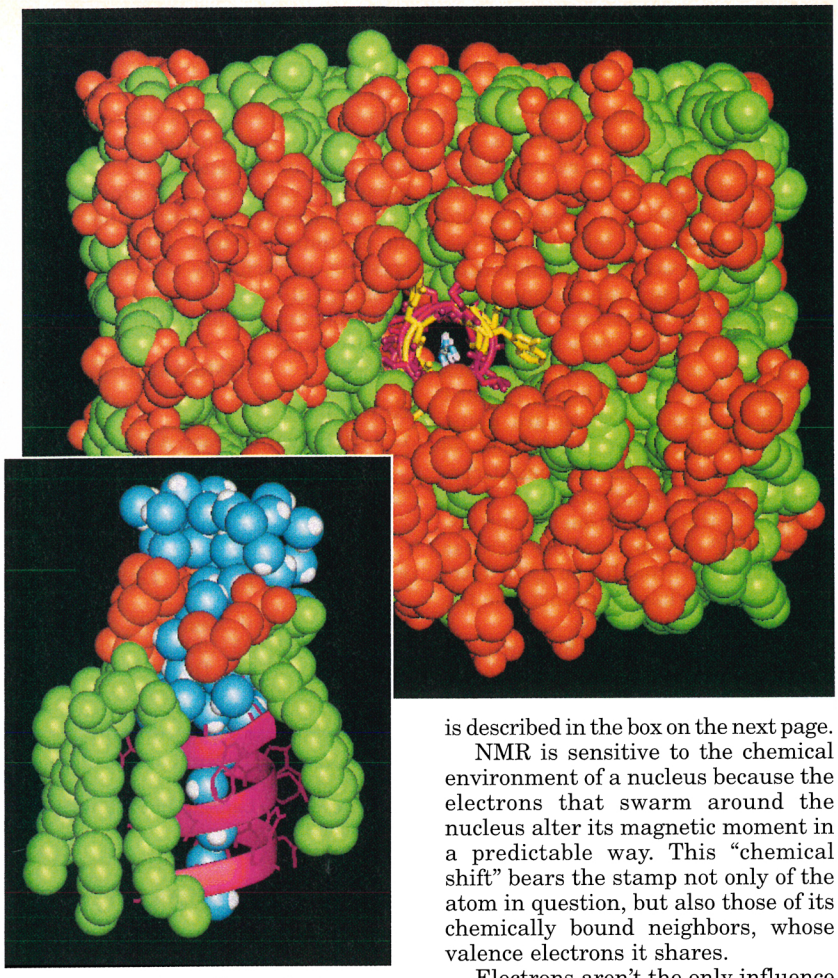
That last disadvantage is unfortunate. Without crystalline samples, x-ray diffraction—the structural biologists' workhorse—can't be used to solve a protein's molecular structure. Of the 20 000 or so solved structures in the Protein Data Bank—a repository for the processing and distribution of three-dimensional macromolecular structure data—only a few handfuls correspond to membrane proteins.

Structure-solving techniques based on nuclear magnetic resonance (NMR) don't require crystalline samples, but they have proved just as challenging as x-ray crystallography to apply to membrane proteins. Recently, however, a significant milestone has been reached in NMR-based structure determination. Two groups—Francesca Marassi and Stan Opella at the University of Pennsylvania[1] and Tim Cross and his collaborators at Florida State University[2]—have independently demonstrated a method that can straightforwardly measure the position and orientation with respect to the membrane of a protein's alpha helices, key elements of the protein's structure.

Although this advance falls short of the ultimate goal of solving the complete structure of an arbitrary membrane protein, "determining the alpha helices' disposition of is," says the University of Cambridge's Richard Henderson, "a magnificent achievement."

Chemical shifts

Unlike crystallography, NMR doesn't provide a picture in reciprocal space of a complete molecule. Rather, NMR data consist of a set of resonance peaks whose properties are shaped by the chemical environment of each



GRAMICIDIN is the first molecule whose structure was determined with solid-state NMR. It's also the smallest known membrane transport protein that forms an ion channel. In the insert, gramicidin's protein backbone is shown as a ribbon and its side chains in a bond representation—both in magenta. Around gramicidin are phospholipid molecules from the surrounding membrane (hydrocarbon chains in green, head groups in red) and water (blue oxygens and white hydrogens). The main panel, in the same color scheme, shows the view from the top of the ion channel. (Courtesy of Eric Jakobsson, University of Illinois at Urbana-Champaign.)

kind of spinning nucleus in the sample. Armed with a protein's amino acid sequence (derived chemically) and sophisticated computer algorithms, NMR practitioners use their data to identify the one stable configuration that the protein invariably adopts.

The figure on this page shows one such NMR-derived structure—gramicidin—that was solved with 144 orientational constraints. How biophysicists use this structural information is described in the box on the next page.

NMR is sensitive to the chemical environment of a nucleus because the electrons that swarm around the nucleus alter its magnetic moment in a predictable way. This "chemical shift" bears the stamp not only of the atom in question, but also those of its chemically bound neighbors, whose valence electrons it shares.

Electrons aren't the only influence on nuclear magnetic moments. Neighboring atoms, if they possess nuclear spins, can interact as magnetic dipoles. In solution NMR, the main NMR technique used for determining molecular structure, dipole–dipole interactions were once regarded as a nuisance because they broaden the NMR resonance peaks. But for small molecules, the Brownian tumbling of molecules in solution effectively cancels the directionally dependent dipolar signal through geometrical averaging. Sharp resonance peaks are the result. The cancellation fails for large molecules, which are too lumbering to visit all rotations on the nanosecond time scales of dipolar interactions.

A different approach, solid-state NMR, is used for the large and insoluble membrane proteins. With fixed, rather than freely tumbling, molecules, the effects of dipolar coupling can't be avoided, but they can be exploited. Dipolar coupling offers a rich source of structural information, thanks to its directional nature and sharp dependence on internuclear separation ($1/r^6$). "The trick," says Opella, "is to come up with NMR pulse sequences that replace molecular motion as a line-narrowing mechanism, but leave the orientation info for structure determination."

In effect, what such pulse sequences do is create a two-dimensional map of the protein, one dimension being chemical shift, the other dipolar coupling. Known generically as separated local field spectroscopy, this pulse-sequence approach was devised in the 1970s by MIT's John Waugh, who showed how peaks in the two dimensions could be associated with specific molecular sites. The early applications were bedeviled by low resolution in the dipolar dimension, a limitation that the Pennsylvania and Florida teams have now overcome.

To demonstrate their method, Marassi and Opella chose to work on key parts of two proteins: the transmembrane helix of the M2 protein that corresponds to the pore-lining segment of the acetylcholine receptor (a neurotransmitter that triggers salivation and muscle contraction) and the membrane surface helix of magainin (a natural antibiotic found in the secretions of certain frogs). Cross and company picked the transmembrane segment of the M2 protein from Influenza A virus (the most frequent cause of influenza).

Alpha helices

Proteins consist of chains of covalently linked amino acids. The links are peptide bonds formed when one amino acid's NH_2 end bonds with another's COOH end. In many proteins, parts of the amino acid chain attract each other to form a helical structure known as an alpha helix: Specifically, the NH of each peptide bond is hydrogen-bonded to the CO of a neighboring peptide bond four peptide bonds away on the same chain.

Alpha helices feature in many membrane transport proteins because, singly or together, they can form a membrane-spanning passage. Additionally, the amino acid side chains provide versatility. The side chains that poke outward, being nonpolar, are compatible with the nonpolar interior of the cell membrane, whereas the inward-poking side chains, through their disposition and polarity, control the molecular transport.

Remarkably, it turns out that the orderly spiral arrangement of amino acids in an alpha helix is directly manifested in the chemical shift–dipolar coupling plane as a bracelet-like feature, as shown in the figure on page 21. Each "pearl" in the bracelet corresponds to the NH half of each peptide bond, whose chemical shift

Gramicidin: from Structure to Function

In 1939, the microbiologist René Dubos discovered that the soil-inhabiting bacterium *Bacillus brevis* could kill pathogenic bacteria of the genus Staphylococcus. Later that year, Dubos isolated the toxin and named it gramicidin.

Efficiency as an ion channel is what gives gramicidin its potent antibiotic effect. When it comes into contact with Staphylococcus, gramicidin breaches its victim's membrane, causing cations to spew outward, fatally depriving the cell of the means to balance its metabolism.

With just 30 amino acids, gramicidin is the smallest molecule that forms an ion channel and was the first membrane transport protein to have its complete structure solved by solid-state NMR (in 1993 by Florida State's Randall Ketchem, Weidong Hu, and Tim Cross). Not surprisingly, researchers who study how ion channels work use gramicidin as a prototype. "We call it the hydrogen atom of ion channels," says Eric Jakobsson, a biophysicist at the University of Illinois at Urbana-Champaign.

As Jakobsson explains, a molecule's structure derived from NMR or crystallography corresponds to the molecule's average configuration. As such, it can't embody the molecule's thermodynamic fluctuations, its dynamic interactions with the membrane lipids and the surrounding aqueous solutions, or the actual passage of the ions or molecules through its channel. In fact, the behavior of the system—membrane, ion channel, ion, solution—is tractable only through computer simulation. As is the case for other complex systems, such as Earth's climate or a supernova explosion, simulating gramicidin in action involves an unavoidable tradeoff between the scales of length and time: The smallest features (electronic orbitals) are modeled in detail with quantum chemistry, but statically, whereas the fastest interactions (such as the response of the local electric field to the passage of an ion) are parameterized and implemented in the model with stochastic dynamics. Molecular dynamics bridges the two regimes.

Basing their models on the NMR-derived structure, Jakobsson and company have discovered that gramicidin is surrounded by a layer of "boundary lipids" whose hydrocarbon chains are more ordered than in the membrane. They also found that the lipids' phospholipid head groups crowd around the mouth of the channel, forming a tortuous pathway for water to funnel from its bulk phase into the protein's narrow channel.

and dipolar coupling both depend on the orientation of the bond with respect to the magnetic field. And the shape of the bracelet as a whole—whether circular or elliptical—is a direct measure of the orientation of the helix. These bracelet-like patterns can be reproduced by spectral simulations.

To obtain the NMR data, bacteria are first coaxed into making the proteins of interest from amino acids in which ^{15}N has been substituted for the naturally more abundant ^{14}N (the quadrupolar ^{14}N nucleus relaxes too quickly for dipolar coupling experiments). Once purified, the proteins are incorporated into artificially created phospholipid membranes, which are layered one on top of the other and held flat between thin glass sheets. Up to 50 of the glass-membrane-glass sandwiches, each containing about 50 membranes, are stacked one on top of the other and oriented so that the membrane is perpendicular to the magnetic field.

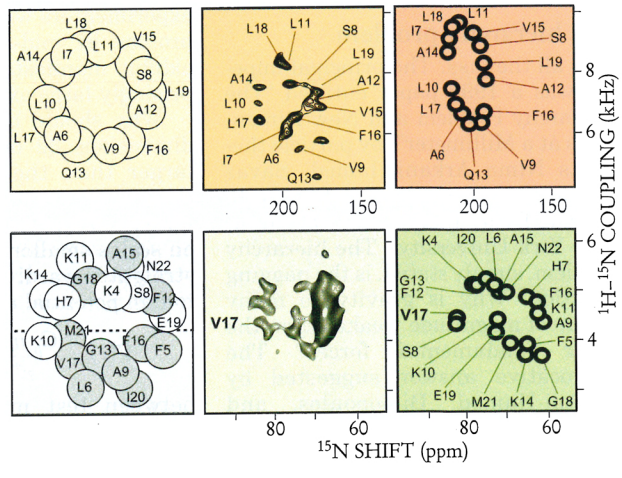
PISEMA

Making a clear two-dimensional map of the alpha helices through the N–H bond requires very careful manipulation of the two nuclear spins involved, ^{15}N and ^{1}H. In particular, the strong dipolar coupling of ^{1}H to its ^{1}H fellows must be suppressed if the much weaker ^{15}N–^{1}H coupling is to be detected with sufficient accuracy.

To achieve that goal, Opella and his colleagues Ayyalusamy Ramamoorthy and Chien Wu devised the following scheme (published six years ago[3]), which the Pennsylvania and Florida groups both took advantage of. The crux of the method is a phase- and frequency-shifted pulse sequence that locks the ^{1}H spins at the so-called magic angle, the angle at which the ^{1}H–^{1}H dipolar coupling goes to zero. At the same time, another set of pulses, synchronous but in antiphase, allows the ^{15}N nuclei to exchange spin with the ^{1}H nuclei. When the spin exchange has stopped, the researchers record the ^{15}N magnetizations, which are modulated by what went on during the spin exchange. Fourier-transforming these temporal data into the frequency domain separates the dipolar and chemical shift dimensions to create the two-dimensional spectra shown in the middle panels of the figure below.

Dubbed PISEMA (polarization inversion spin exchange at the magic angle), this sequence greatly enhances the dipolar signal of the ^{15}N–^{1}H bonds because it extends the corresponding oscillations in time, thereby sharpening the frequency peaks. In effect, PISEMA forces the ^{15}N–^{1}H coupling to decay on the rotating-frame spin–lattice time scale, rather than the faster spin–spin time scale.

If you know the orientation of a protein's alpha helices, how much can you tell about the protein's function? "Both a lot and a little," says Tom Woolf of Johns Hopkins University. "The partial structural information provides important clues for design of mutagenesis experiments and suggests some aspects of function. But it does stop well short of a full molecular idea of the 'workings' of a membrane protein." Woolf also cautions



TWO-DIMENSIONAL NMR SPECTRA of the transmembrane helix of the M2 protein that corresponds to the pore-lining segment of the acetylcholine receptor (top row) and the membrane surface helix of magainin (bottom row). The two panels on the left show the arrangement of amino acids (for example, "L18" denotes leucine as the 18th amino acid). The middle two panels show the actual two-dimensional NMR spectra in which each amino acid occupies a definite and identifiable locus. Panels on the right are computer simulations of the spectra. (Adapted from ref. 1.)

that all the current structure-determining methods are hampered somewhat by the difficulty in manufacturing sufficient amounts of protein. But, as stronger and stronger magnets become available, NMR methods become more sensitive, making it possible to use smaller samples. Higher magnetic fields will also add another arrow to the solid-state NMR quiver: the ability to exploit the ^{1}H chemical shift as a third, orientation-constraining dimension.

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Theorists and Experimenters Seek to Learn Why Gravity Is So Weak

At the recent Marcel Grossmann Meeting on General Relativity in Rome, Jens Gundlach of the University of Washington's Eot-Wash laboratory reported a provisional result from the group's examination of gravity at submillimeter distances. At distances as small as 0.2 mm, he said, the group's specially designed torsion balance has not, as yet, revealed any departure from Newtonian $1/r^2$ gravity.

Just a few years ago, this result might have elicited little more than yawns. Why, after all, should one doubt that Newtonian gravity holds at such macroscopic distances? Admittedly, Cavendish-type experiments had not been able to test the gravitational force at separations smaller than a millimeter. But surely that was only the concern of specialists obsessed with checking things that most of us take for granted.

Nowadays, however, it's all different. In the past two years, testing gravity at submillimeter distances has become a cottage industry. The Eot-Wash group (whose name is a play on that of Baron Roland von Eötvös, who tested the equivalence principle with a torsion balance a century ago) is but one of perhaps a dozen groups that have recently set out to look for departures from Newtonian gravity at these small but macroscopic distances. Their results are eagerly awaited.

The hierarchy problem

Why all the fuss? The principal impetus was a 1998 paper entitled "The Hierarchy Problem and New Dimensions at a Millimeter"[1] by particle theorists Nima Arkani-Hamed, Savas Dimopoulos (both then at Stanford), and Gia Dvali (then at Trieste, now at New York University). The hierarchy problem, simply stated, is the nagging question: Why is gravity so many orders of magnitude weaker than the other fundamental forces? The provocative answer suggested by Arkani-Hamed, Dimopoulos, and Dvali (ADD) supposes the existence of two or more as yet undetected spatial dimensions, in addition to the four dimensions of ordinary spacetime.

> If gravity leaks out into macroscopic extra dimensions, we may soon find departures from the inverse-square law at millimeter separations.

String theorists have long since inured us to the notion of half a dozen extra dimensions, unseen because they are presumed to be curled up ("compactified," as they say) into loops about 10^{-33} cm in diameter. That's the so-called Planck length L_P, the distance at which, in standard particle theory, gravity finally becomes equal to the strengths of the other forces. Examining such absurdly tiny distances would require probe energies of order 10^{19} GeV, the "Planck mass" $M_P = \hbar/L_P c$, far beyond the capabilities of any conceivable accelerator. (Specifically, M_P is the mass at which a particle's Compton wavelength becomes equal to its Schwarzschild radius.)

But ADD were enticing experimenters with much more accessible prospects. They argued that the extra dimensions might be curled up on a scale as large as a few *millimeters*, making it possible to detect departures from Newtonian gravity with a new generation of sensitive tabletop experiments. Furthermore, they pointed out, the Large Hadron Collider (LHC), which will be providing experimenters with 10 TeV (10^4 GeV) protons by mid-decade, should also exhibit manifestations of these surprisingly large extra dimensions.

Why should one believe in extra dimensions 32 orders of magnitude larger than the Planck length? If there are n extra dimensions curled up with diameters R, anyone looking on scales smaller than R would see a straightforwardly generalized Newtonian potential energy

$$V(r) = \frac{G_n^* m_1 m_2}{r^{n+1}} \quad \text{for } r \ll R \quad (1)$$

between test masses m_1 and m_2, where G_n^* is the appropriate gravitational constant for n extra dimensions. Gravity, because of its intimate relation to the fabric of spacetime, must spread out in all the dimensions. And the extra dimensions make the gravitational force grow faster with decreasing separation. But if you're only looking at scales *larger* than R, you would see a Newton-like potential

$$V(r) = \frac{G_n^* m_1 m_2}{R^n} \frac{1}{r} \quad \text{for } r \gg R. \quad (2)$$

Long before the Planck scale

In natural units ($\hbar = c = 1$), Newton's constant G is essentially L_P^2, or equivalently, $1/M_P^2$. The central point made by ADD is that a real $4 + n$ dimensional gravity would become equal to the other fundamental forces long before the remote Planck scale. This unification, they suggest, occurs at the same modest length scale $L_{ew} \approx 10^{-17}$ cm at which electromagnetism is unified with the weak nuclear force (and the strong nuclear force is not far off). In other words, the implausible, yawning chasm between electroweak unification and the Planck scale is abolished. The electroweak distance scale, corresponding to a mass M_{ew} of about 1 TeV, becomes the only unification scale, and the hierarchy problem is gone.

What does this tell us about the size R of the compactified extra dimensions necessary to make the trick work? If there are n extra dimensions and the fundamental unification scale of gravity is L_{ew}, then the true coupling constant G_n^* in equation 1 is (again in natural units) L_{ew}^{2+n}. So equation 2 tells us that the familiar Newton's constant G we've been measuring at separations larger than R is really $G_n^*/R^n = L_{ew}^2 (L_{ew}/R)^n$. In effect, gravity is intrinsically comparable to the electroweak forces. Only its leakage into the extra dimensions makes it appear so much weaker to us. And the compactification size of the n curled-up dimensions is given by

$$R^n = L_{ew}^n \left(\frac{L_{ew}}{L_P}\right)^2 \approx L_{ew}^n \times 10^{32}. \quad (3)$$