

## Breaching the Membrane

A better understanding of the structure and function of the cell membrane and its components is providing drug developers with new avenues for breaching the cell's outer defenses to deliver drugs or DNA

The cell membrane has a tough job. A mere 7 to 10 nanometers thick, this jittery association of lipids and proteins has two seemingly incompatible responsibilities: to prevent the inside and outside of a cell from mixing and yet allow specific molecules to enter the cell and others to exit. Failure to carry out either mission means certain death for a cell. But success can also have its negative consequences when the cell membrane bars entry to a drug searching for its therapeutic target.

"Getting a potential drug past the cell membrane to reach its target is a huge challenge and one that we often fail at," says Gordon Amidon, a pharmaceutical chemist at the University of Michigan, Ann Arbor. "And even when we have succeeded, it's largely been because of serendipity, not because we actually understood how to do it."

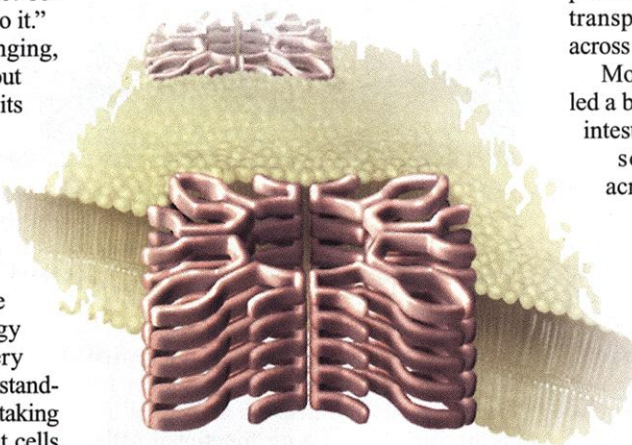
That sorry state of affairs is changing, though, as researchers learn more about how the cell uses the membrane and its protein components to admit some molecules while excluding others. Already, one drug designed to take advantage of a specific transport mechanism has reached the market—the antiviral agent valganciclovir, used to treat a potentially blinding eye infection—and several biotechnology start-ups are developing drug-delivery technologies based on the new understanding of cross-membrane transport. "By taking advantage of the very mechanisms that cells use to take up or exclude certain molecules, we're making drug delivery a rational science," says Ronald Barrett, co-founder and chief executive officer of XenoPort in Santa Clara, California.

Bringing rationality to what has been a hit-and-miss proposition will have tremendous implications for drug development. For example, many of these membrane-bound molecular transporters are unique to particular organs, which could eventually lead to tissue-specific drug delivery, long a goal of medicinal chemists. And recent work in Amidon's laboratory shows that tumor cell lines contain a collection of transporters different from those of healthy cells, which raises the hope of targeting tumors through their transporters. "This field has made such rapid progress that we're only just starting to

imagine all the ways we can use cell-specific transport mechanisms in medicine," says environmental toxicologist Ned Ballatori of the University of Rochester School of Medicine in New York state, who has been studying how transporters help clear the body of toxic chemicals.

### Hijacking the transporters

Plenty of places in the body are for all intents and purposes unreachable, at least as far as many potential drugs are concerned. The vast majority of drugs that are now injected, such as the antibiotic vancomycin, cannot break through the membrane of the cells that line the digestive system, which means they cannot be taken in pill form.



**Tunneling in.** Rather than use an existing transporter, a group at Scripps is constructing artificial passageways through the cell membrane using self-assembling peptide nanotubes.

Others never make it out of the bloodstream or past the tightly packed band of cells known as the blood-brain barrier. In each case, the largely impermeable cell membrane is the culprit. Those molecules that do cross the membrane, such as necessary nutrients and hormones, do so courtesy of transporters embedded in the membrane—molecular portals, if you will, that ferry in molecules meeting specific criteria for size, charge, or chemical composition.

Hijacking a transporter seems a promising way to get a drug across the cell mem-

brane, "but being able to do that assumes that you know a great deal about these carrier proteins, including their distribution on various organs and cell types and their specificity [for particular molecules]," notes Amidon. That's clearly not the case. But fortunately for drug developers, most individual transporters fall into distinct families that share many functional features. For example, two types of transporters, known as PEPT1 and PEPT2, are responsible for ferrying a broad range of nutrients across a variety of cell membranes. Serendipitously, a variety of drugs, including ACE inhibitors and  $\beta$ -lactam antibiotics, can use PEPT1, something that only became known over the past few years.

With nary a single crystal structure available for any transporter, researchers have taken two approaches to characterizing these proteins. One line of attack, taken by biophysicist and pharmaceutical scientist Peter Swaan of Ohio State University, Columbus, and others, is to use site-directed mutagenesis, binding data, and computational techniques to create in silico models of a transporter. Swaan's group, for example, has modeled the binding site of the PEPT1 transporter, creating a tool that can predict those molecules that this particular transporter will accept as cargo to ferry across the membrane.

More recently, Swaan's group has tackled a bile acid transporter, a high-throughput intestinal transporter capable of shuttling some 10,000 molecules a second across the membrane. Starting with the known structure of the distantly related membrane protein bacteriorhodopsin as a digital scaffold, the researchers created a structural model that identified five unique binding domains at which specific molecules bind depending on their chemical structure and polarity. Swaan says his group has successfully

used this model to modify several molecules so they can slip into cells through the bile acid transporter.

A second approach, taken by Amidon's group and others, has been to synthesize hundreds of molecules to determine the kinds of chemical groups that members of PEPT1 will carry across the membrane using an in vitro model system. With this information in hand, the Michigan team created so-called prodrugs—a chemically modified form of drug that gets converted into the active form by enzymes present in a cell—of the antiviral agents acyclovir and AZT that increased the intestinal absorption of these drugs from three- to 10-fold. The prodrug consists of the parent antiviral coupled with a hydrolysable chemical link to

the transportable group. The transporter recognizes its target group and drags it and the rest of the prodrug through the membrane. Ubiquitous enzymes inside the cell cleave the linkage, yielding the active drug. Chemists at drug company Roche used the same approach to make valganciclovir, a transportable prodrug of the antiviral agent ganciclovir that requires less than one-tenth as much active drug to achieve the same therapeutic effect.

One of the holy grails of drug development is to create therapeutics that only act where they are needed in the body. XenoPort scientists believe they know how to find this grail—by mapping where one widespread class of transporters is distributed in the body. Such a map would enable medicinal chemists to select a particular transporter to target in order to deliver a potential drug to a specific tissue. So far, company scientists have studied over 200 transporters and shown that many are found in specific locations in the body. Using combinatorial chemistry techniques, the XenoPort team hopes eventually to determine what kind of molecules each of these transporters will accept as cargo. For now, though, the focus is on those found in the intestines, with the aim of improving the absorption of drugs as they pass through the intestinal tract. XenoPort researchers have, in fact, identified the chemical tags needed to gain passage through some of these intestinal transporters.

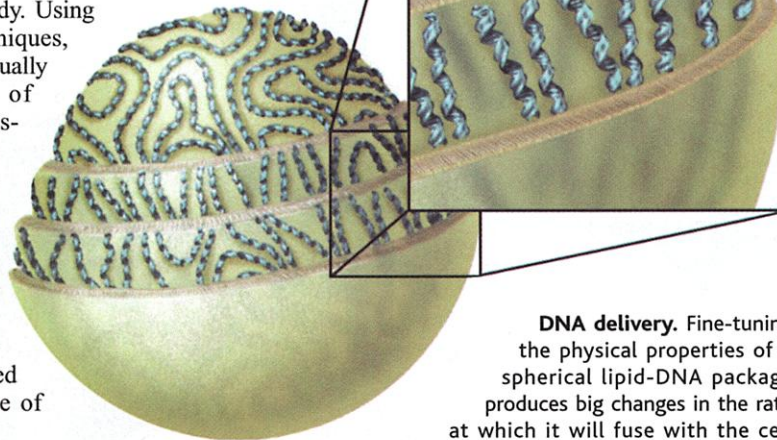
#### Breaking and entering

Rather than rely solely on existing transport systems, some investigators are choosing to make a new pathway through the cell membrane. M. Reza Ghadiri and his colleagues at the Scripps Research Institute in La Jolla, California, have created their own transporters using self-assembling peptide nanotubes that can insert themselves into the cell membrane. In the group's most recently published work, which appeared last year in *Angewandte Chemie*, a large ring made of 10 amino acids—five D-leucines and L-tryptophan—selectively transported glutamic acid across an artificial cell membrane. The process was not very efficient, Ghadiri concedes, but since then, he says, his group has made significant progress in designing more efficient transporters for molecules substantially more complex than glutamic acid. "We feel we're very close to designing transporters specifically for drug delivery," he explains.

Cyrus Safinya, a condensed matter

physicist at the University of California, Santa Barbara, thinks he can beat nature at its own game by understanding the chemical nature of the membrane itself. Working with colleagues Nelle Slack, Alison Lin, Ayesha Ahmad, Kai Ewert, and Heather Evans, Safinya has been studying the complexes that form between positively charged, or cationic, liposomes and DNA—a hot technology for delivering genes into cells for gene therapy. "Clinicians use these cationic liposomes in a trial-and-error manner with little idea of why one liposome is better than another at getting DNA across the membrane," says Safinya.

Disdaining blind luck, Safinya's group has worked out the molecular details of how a cell membrane and a



**DNA delivery.** Fine-tuning the physical properties of a spherical lipid-DNA package produces big changes in the rate at which it will fuse with the cell membrane and deliver its cargo.

liposome membrane will best fuse with one another. Using a variety of x-ray diffraction techniques, the group determined that DNA and cationic lipids form primarily a sandwich structure with DNA layered between the cationic lipids; on rare occasions they form an inverted hexagonal structure with DNA encapsulated in lipid tubules. Recent studies have begun to unravel the relation between these distinct nanostructured supramolecular assemblies and how effectively DNA gets transported, or transfected, into cells. "We found that by tuning key physical and chemical parameters of the lipid carrier, we are able to controllably vary and increase the transfection efficiency by a factor of 10,000 in an *in vitro* model," says Safinya. In fact, Safinya's group can tune the chemical properties of a liposome to get a desired delivery rate into a cell for a particular gene or set of genes.

Chemist Steven Regen of Lehigh University in Bethlehem, Pennsylvania, is

taking a different stealth approach to ferrying drugs—and perhaps DNA—across the cell membrane. He has created a series of molecular umbrellas that can fold around a charged or polar drug and shield it from the cell membrane, allowing the drug to pass through the membrane. The ribs of the umbrella are made of rigid facial amphiphiles, molecules that have separate hydrophobic and hydrophilic faces: The hydrophobic face serves as a membrane-friendly surface, whereas the hydrophilic face provides a hospitable hiding place for the drug molecule.

Regen's group couples two or more of these long molecules to the top of the umbrella shaft and the drug molecule to the bottom of the shaft. In the watery environment outside the cell, the umbrella is open, but as the construct makes contact with the cell membrane, the umbrella begins closing around the drug. The hydrophilic side of the ribs, facing in toward the handle, shields the hydrophilic drug molecule, whereas the hydrophobic side of the ribs slides easily into the cell membrane, where it can immerse itself in the membrane's water-free interior. As the fatty acids that make up the membrane rearrange themselves, they push the umbrella into the cell's aqueous interior, where it opens, making the handle available to enzymes that then release the drug molecule from the umbrella shaft.

In work published in the *Journal of the American Chemical Society* last year, Regen's group showed that one such molecular umbrella was able to carry glutathione across a liposomal membrane and release it into the aqueous interior. The group has also shown that an umbrella containing cholic acid amphiphiles can ferry highly covalently attached nucleotide bases across a liposome membrane, the first step toward delivering therapeutic DNA and RNA across the cell membrane. "In our test systems, these umbrellas seem quite versatile," says Regen. "Now we have to put them into animals and see if they can deliver drugs." That's going to be the bottom line for all of these approaches.

—JOE ALPER

Joe Alper is a writer in Louisville, Colorado.