Profile of Francisco Bezanilla

In the 1950s and 1960s, most electronics hobbyists learned the trade by building radios. Francisco Bezanilla went a step further: he made his own television. Bezanilla grew up in Santiago, Chile, and when Chile hosted the World Cup in 1962, the country broadcast soccer games around the globe from new transmitters installed on the campuses of the national universities. Television sets were extremely rare, so Bezanilla and a friend decided to build their own. "We even had to design the coils of the intermediate frequency amplifiers," he says. "There was a table full of electronics and a little oscilloscope tube for a screen."

Bezanilla's parents dreamed of watching Brazil's Pele perform his magic, in flickering green and black if necessary. Unfortunately, the "television" was completed shortly after the World Cup's final games. Later, Bezanilla's parents sent their son to Argentina to buy commercial parts, out of which he fashioned a new set that they used for many years.

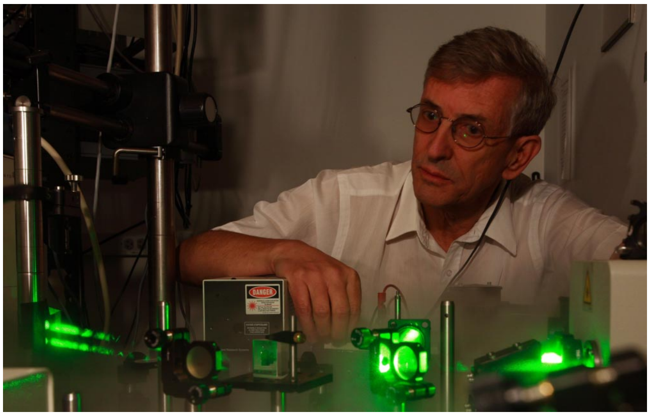
Bezanilla, whose intimate knowledge of electronic hardware was acquired during his childhood, is now a biophysicist whose advanced experiments on neurons and ion channels earned his election to the National Academy of Sciences in 2006. In addition to his work on ion channel gating, Bezanilla is a pathfinder in the use of fluorescent labeling techniques for studying dynamic rearrangement in channel proteins, and codiscoverer of gating current in sodium channels.

In his Inaugural Article, published in this issue of PNAS (1), Bezanilla reports on ion channels.

Although Bezanilla concedes that his research on the molecular basis of voltage sensing does not have direct application in any one particular area, it is fundamentally important to many fields. "It is the basis of the nerve impulse, the heart beating, brain activity," he says. "Even enzymes are activated by voltage."

Certain genetic abnormalities affect voltage sensing, such as the "long QT syndrome," a sometimes-fatal heart condition characterized by arrhythmias and associated with exercise or excitement. "People exercising can suddenly drop dead because there is a problem with the repolarization of the action potential of the heart," he says. His research could lead to the development of new drugs to treat long QT syndrome and related conditions.

Bezanilla recognizes that his expertise with electronics has been key to his research success and wishes that young scientists today had a deeper understanding of the instruments they employ. "Today, people doing patch clamping just buy the system with a computer and everything. They run the program. But they have very little idea what is going on behind the scenes."



Francisco Bezanilla

This is a problem, he says, because the complex equipment can generate artifacts that a novice might think represent an experimental result. To ground his own graduate students in the basics, Bezanilla, along with Julio Vergara, initiated a special course in electronics at the University of California, Los Angeles (UCLA, Los Angeles, CA), that he and Vergara taught for a quarter of a century and is still offered today.

"If you look at the history of physiology," Bezanilla says, "every big advance came after somebody invented some new equipment." He knows of what he speaks; his first major discovery occurred after he designed and built a device to measure ion channel gating currents superior to any on the market at the time.

The Escape Hatch to Montemar

At the Catholic University of Chile (Santiago, Chile), Bezanilla earned a bachelor's degree in biology, which led him into the university's School of Medicine. He was not set on becoming an M.D., however, and sought an escape hatch: after his third year of medical studies, he took an option that allowed him to try his hand at research.

"Of course, when I got into neurophysiology, that really was it," he says. "It was so clear that that's what I wanted, because it joined biology with electronics." He picked up classes in physics, chemistry, and math in the School of Engineering, and completed his Ph.D. at Montemar, the world-famous neurophysiology laboratory on the Chilean coast near Valparaiso. His advisor was Eduardo Rojas.

Rojas and the other scientists at Montemar were legendary for their ability to improvise experiments with spare parts. What made Montemar a magnet for researchers was the easy access to inexpensive boatloads of *Dosidicus gigas* (also known as the Humboldt squid), which can reach two meters in length and has "giant axons" twice the diameter of its Atlantic relative, *Loligo pealeii* (or longfin inshore squid). *L. pealeii* is the experimental mainstay of neurophysiologists in the United States. The size of the *D. gigas* giant axon, however, makes it easier to insert electrodes, and the larger surface area makes the signal-to-noise ratio extremely high. Bezanilla completed his dissertation on the giant axon's action potential—the electrical neuron-firing impulse—in 1968.

At Montemar, Bezanilla met many American scientists, including Robert Taylor and Clay Armstrong. Both men, regular visitors to Montemar during the southern hemisphere summers, would be mentors and collaborators of Bezanilla's in the years to come. Taylor, who worked at the National Institutes of Health in Bethesda, MD, was impressed with Bezanilla and invited him to his laboratory for a postdoctoral fellowship. Bezanilla followed this stint with a second postdoc in the laboratory of Paul Horowicz, chair of the Department of Physiology at the University of Rochester (Rochester, NY), where Armstrong held an appointment.

Exit the Squid

In 1970, while Bezanilla was at National Institutes of Health, he heard surprising news: the Humboldt squid had mysteriously disappeared from the coast of Chile. The fishermen could not catch them for market, and the scientists had lost their source of giant axons. No one knew where

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 17600.

Francisco Bezanilla on the summit of Maparaju, Peru.

the squid had gone. The foreign scientists stayed home; the research at the beachfront lab at Montemar switched focus, to barnacle giant muscle fibers. The ascent of Pinochet's military government made times even bleaker for Montemar, which was finally abandoned in 1990.

D. gigas and its monster nerve fibers were, it seemed, a memory—until 2005, when Bezanilla heard from his Chilean colleague Miguel Holmgren that the squid had come back.

In January 2008, he and Holmgren revisited Montemar, now dusty and dilapidated, to clean up their old lab and begin a round of new experiments. "People are quite puzzled" about the squid's migration, Bezanilla says, although he recalls once finding *D. gigas* mentioned in an American textbook from the 1930s. "Found between San Diego and Monterey on the Pacific coast of the United States," it said. He thinks the squid cycle back and forth between the two regions every 30 years or so.

In 1972, Armstrong invited Bezanilla, then a postdoc in the Horowicz lab, to work at the Woods Hole Marine Biological Laboratory (Woods Hole, MA) center for the summer, where scientists dissected *L. pealeii* instead of *D. gigas*. "It started a fantastic collaboration," Bezanilla says. "We began by trying to block potassium channels with sodium. And then we made the first attempts to measure gating currents."

Tiny Gating Currents

The concept of a gating current is simple enough: charged amino acid residues of an ion channel's voltage sensor move under the impetus of an external field. The moving charges constitute a current, which induces a compensatory current in a voltage clamp experiment that a scientist can measure.

Ion channels are subtle and complex, however. Once the voltage sensor has moved, it is much more likely, but not absolutely certain, that the gate of the ion channel will open. And there are many varieties of ion channels—sodium, potassium, calcium, proton—and many of them possess voltage sensing domains that are essentially identical in structure. Bezanilla has spent his career contemplating how voltage sensors gate ion channels.

Bezanilla and Armstrong were the first to succeed in measuring gating currents of

"[Voltage sensing] is the basis of the nerve impulse, the heart beating, and brain activity."

the sodium channel. They faced the challenge of measuring a tiny signal that is normally swamped by much larger currents when the voltage difference changes across a membrane.

Normally the inside of the cell is at approximately −60 mV potential compared with the outside; there are many more sodium ions outside than inside, whereas the opposite holds true for potassium ions. When the potential difference changes, as in the case of an action potential zipping down a squid axon, the voltage sensors shift—this is the gating current—and then sodium ions rush in, potassium flows out, and at the same time trapped "capacitive" charges on the membrane switch sides.

"It's very hard to see gating currents," Bezanilla says; they are about one-200th the size of the ionic and capacitive currents. He and Armstrong designed a method to subtract the larger signals.

Naturally, it was not a simple task. "We borrowed a signal averager to eliminate noise," Bezanilla says. "We did all of the experiments. We got results that indicated we could really do it."

But the summer ended and they had to return to Rochester. There, he and Armstrong designed and built a new signal averager. "The ones that were commercially available, they were not at the speed we needed." There was an extra challenge: digital components had only just entered the scene, and Bezanilla found himself suddenly working with flip-flops, memory, and digital-to-analog converters. The device completed, the two returned to Woods Hole the next summer to resume their experiments.

Shorn of the capacitive and ionic currents, the minuscule gating currents of sodium channels stood revealed. Bezanilla and Armstrong published their results in *Nature* (2).

"What I learned from this," Bezanilla says, "was that if you can design something, or modify something that is available, you can really do new things, which were not possible before."

California Bound

Before the completion of his work with Armstrong, he had accepted, but deferred, a professorship at the University of Chile (Santiago, Chile). Now, he returned to his home country, although he rejoined Armstrong at Woods Hole for northern hemisphere summers. The Pinochet government gave him no trouble as he traveled back and forth. "It was good," he says, "because that way I could work with Clay and, at the same time, bring parts to build stuff in Chile to maintain some of the research."

He did his best, but the 1970s were a hard decade for Chile. Without funding or support, Bezanilla found the situation untenable. He left for good in 1976, taking his wife and two children—both of whom were born in the United States. After a year as a visiting professor at the University of Pennsylvania (Philadelphia, PA), he took at permanent position at UCLA.

"California and Los Angeles, it's very nice, like Chile," he says. "Like a mirror image in this part of the Pacific. I felt at home there."

Initially, Bezanilla tried to obtain some local squid but the species were too small. "We spent most of the time collecting specimens and keeping them alive," he says. But because this effort was not paying off, Bezanilla shuttled his entire lab from UCLA to Woods Hole by truck in the summers to work with *L. pealeii* axons. "Every year from 1980 to 1992," he says. "There were nine crates of equipment."

One important result that came from Bezanilla's work at Woods Hole was that he, along with Michael White and Robert Taylor, were able to identify the gating currents associated with the opening of potassium channels in the squid axon.

Sodium channels open much more quickly than potassium channels, and their gating current, although hidden by the capacitive and ionic currents, is more easily visible.

Bezanilla and colleagues sought to find a slower component of the gating current that would correspond to the voltage sensors in the potassium channels. They were able to do this, it turned out, by conducting their experiments at warmer temperatures that sped up the action of the potassium channels (3).

Changing Boundaries of Possibility

By 1992 it was clear that molecular biology had expanded the boundaries of neurophysiology. By making mutant versions of the proteins, researchers could explore the importance of different regions to the channels' operation, or as Bezanilla puts it, to "see what part of the channel is doing what."

"We'd made a lot of progress in terms of gating currents and how their kinetics are related to the opening of the channel," Bezanilla says, "but then came the possibility of cloning the channels. We didn't need the squid any more."

At his fingertips now were Shaker potassium channel genes from fruit flies, which he and colleagues expressed in frog eggs for voltage clamp experiments. The Shaker channels close spontaneously soon after opening when the cells are depolarized.

Researchers had suspected that a certain domain of the protein automatically swung into place after the channel opened, in a form of timed shutoff. Bezanilla and colleagues found that this "inactivation" domain interacted with the voltage sensor part of the channel and prevented it from returning to its original position, thus blocking the channel (4).

The techniques of molecular biology allowed Bezanilla not only to delete sections of channel proteins but also to modify individual amino acids. In an experiment that spanned physics and biology, drawing on the extremes of his expertise, he substituted cysteine residues at locations on the voltage sensor of a potassium channel. The cysteines allowed him to attach reactive fluorophores and, using spectroscopy to measure the resonant transfer of energy between fluorophores, observe movement of the voltage sensor on the atomic scale (5).

One of the most interesting things he has discovered in the course of his mutation work, Bezanilla says, is that the electric field is not uniform in the region of an ion channel (6).

"It is shaped in such a way that it's concentrated near the charges of the voltage sensor," he says. "When the charges move, they don't need to move very far to have an effect."

Lured to Chicago

As much as he enjoyed UCLA, Bezanilla was lured away in 2005 to the University of Chicago (Chicago, IL). Steve Goldstein, an ion channel specialist, had just been appointed chairman of the Department of Pediatrics and was recruiting experts for a group called Molecular Pediatrics Science. He called up Bezanilla, Eduardo Perozo, a former student of Bezanilla's, Benoît Roux, a theoretician with whom Bezanilla had collaborated for many years, and Ana M. Correa, and made them attractive offers.

"It was like a self-catalyzed process," Bezanilla says, "because Eduardo was asking me 'are you going?' and then Benoît, 'are you going?' and finally the four of us were here. And we are very happy."

Molecular Pediatrics Science was dissolved as a formal group in 2007 and Bezanilla is now officially a professor in the Department of Biochemistry and Molecular Biology.

Finding Fluorescent Tracers

The phenomenon of voltage sensing is not confined to ion channels. Other types of proteins have acquired voltage-sensing modules, or evolved from their origins as ion channels to perform other functions.

The sodium-potassium pump, which creates the ionic gradients necessary for neurons to operate, is voltage-sensitive. Bezanilla's and Miguel Holgrem's visit to Montemar in January 2008 was, in fact, to investigate this pump.

"The squid axon has such a high density of sodium-potassium pumps," Bezanilla says, "and the fastest voltage clamp you can make is still the squid axon."

Another major area of Bezanilla's current research is the voltage-dependent phosphatase Ci-VSP. Ci-VSP is a membrane-bound enzyme that does not possess a conducting pore, but nevertheless has an S4 voltage-sensing domain, a transmembrane sensor commonly found in ion channels.

In his Inaugural Article, Bezanilla reports that the Ci-VSP S4 domain shares the hysteresis behavior seen in ion channels. His research reports that how the S4 domain moves under the influence of an external field depends on whether the potential difference across the membrane has been held for a long time at a negative or positive value. Ci-VSP is not an ion channel, so the hysteresis appears to be a property of the voltage sensor itself, according to the researchers.

In the study, Bezanilla and colleagues traced the movement of fluorophores attached to the sensor domain, and found that there are two kinetic phases. In the first, the fluorescence corresponds to the position of the charge as computed from the gating current. But the second phase of the fluorescence motion is independent of the gating current, they found.

Bezanilla and colleagues propose that the first phase corresponds to the sensor moving from a resting state to the active state, whereas the second, slower phase corresponds to the sensor entering a relaxed state.

In addition, they believe that the S4 segment changes its secondary structure during the process—that it adopts a "3_{10} helix" structure in the brief shift from resting to active, and then becomes an α-helix in the relaxed state, which corresponds to the structure determined by X-ray crystallography. "We will have to use spectroscopic techniques to confirm this," Bezanilla says, "because you have to resolve it on the millisecond time scale."

For Francisco Bezanilla, the deepest satisfaction in his career has come not from a single experimental success, but from a sense that, through his and his colleagues' work, the field of neurophysiology has made tremendous advances in understanding and scope.

"At the beginning, we were working with a black box," he says. "We applied a pulse, recorded a current. And we could characterize that in beautiful detail. And then as time went on, we found what was inside the black box. What the components are, to the point that we know exactly which are the amino acids that are responsible for the gating currents. And that has been very rewarding."

Kaspar Mossman, *Science Writer*

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