Picture credits: Gray group. Are they published structures?

Misfolded Proteins and Parkinson's Disease

by Jay Winkler

Amino acids share a common backbone. To this is attached a side group, or residue, which can be as simple as the single hydrogen atom in glycine. The other atoms are carbon (black), nitrogen (blue), oxygen (red), and sulfur (yellow). Amino acids link up when the acid carbon (the C-terminus) and the base nitrogen (the N-terminus) react, ejecting a water molecule in the process. A complex interplay of forces between the residues—nonpolar and polar, acidic and basic—creates such shapes as this bundle of α-helices, the barrel made of a rolled-up β-sheet, and eventually such complex structures as this photosynthetic reaction site from the bacterium *Rhodobacter sphaeroides*. (The colors in those structures stand for the various amino acids.)

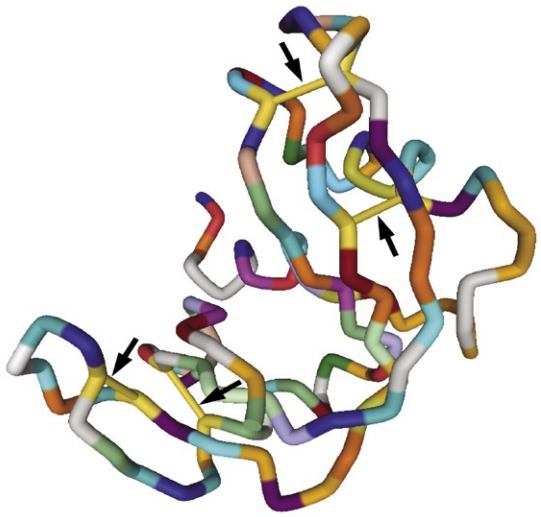
Proteins need to be folded into their correct shapes in order to do their jobs. The folding process is very complex, and there are innumerable ways in which it can go wrong, yet cells do it with a pretty high degree of reliability. How they do so is a very hot field of research, as you might imagine. Here at the Beckman Institute Laser Resource Center we've been developing methods for studying misfolded proteins, and we're very interested in one protein in particular, α-synuclein, that has a direct relationship to Parkinson's disease. But before we start talking about misfolding, we need to talk a little bit about proteins in general, and why proper folding is so important.

The story starts with DNA, which most people know from *CSI* and Court TV—very few molecules get their own shows, much less an entire cable channel. DNA carries genetic information from one generation to the next, and from one cell to another. It carries that information encoded in a sequence of four organic bases, or "letters," arranged like rungs on a ladder that's twisted into the familiar double helix. The code contains "words," each three letters long, standing for the 20 naturally occurring amino acids from which proteins are made. In general, information flows from DNA to RNA—a molecule much like DNA, but single-stranded. The words that go into an RNA molecule are determined by the sequence of letters in the DNA, and the cell uses the information in the RNA to make the thousands of different proteins each cell contains.

Proteins called enzymes catalyze most of the cell's really important chemical reactions, and an enzyme's function is determined by its three-dimensional structure. That structure comes from the amino acids, which a cellular machine called a ribosome—itself an assembly of proteins—strings together in the order prescribed by the RNA. The amino acids have a common backbone that allows them to link to other amino acids, but they all have different shapes—some are small, some are big and bulky; some have floppy side chains, some are rigid. They have different chemical properties as well—some are acidic, some basic; some are polar, some aren't; some have bonding sites, some don't. A complex interplay of forces between these shapes and properties makes the protein fold up in a unique way that is determined by the amino-acid sequence. Enzymes called chaperones often assist the process, but not always. Small proteins in particular can fold completely unaided.

There are roughly 600 general classes of protein structures, and a few fundamental motifs. One common motif is a coil called an α-helix. These coils can form bundles, which can also be helical. Helices are often used as a sort of scaffolding to hold other parts of the protein in position. Another common motif has several protein strands lining up to form a β-pleated sheet. (A single strand in this configuration is called, not surprisingly, a β-strand or β-ribbon.) The sheets often help define the shapes of reactive sites, and they can even wrap around and form barrels. Really complex structures occur when you build an enzyme to be inserted into a membrane that separates different compartments within the cell, or separates the cell from the outside world. Many different proteins are anchored to the membrane, and some actually penetrate it. Typically you find helices spanning the membrane to act as anchors, and then on the inside or on the outside you find a complex structure that includes β-sheets, α-helices, and other things.

In 1972, Christian Anfinsen of the National Institutes of Health won one-half of the Nobel Prize in Chemistry for showing that all the information needed to fold a protein correctly is contained in its sequence of amino acids, which has in turn been coded by the DNA. The other half of the prize was shared by Stanford Moore and William Stein of Rockefeller University, who proved that a protein's catalytic activity is determined by the details of its three-dimensional structure. All



The folded structure of ribonuclease, shown shorn of its side chains for clarity. Again, the colors correspond to the different amino acids. The arrows point to the four disulfide bonds.

three men did their work on a protein called ribonuclease.

Ribonuclease has four cross-linkings, called disulfide bonds, linking two cysteines (a sulfur-containing amino acid) each in widely separated places on the amino-acid chain. In the 1950s, Anfinsen found that he could treat ribonuclease with two chemicals that disrupted its structure entirely. One of them, mercaptoethanol, broke the disulfide linkages and the other, urea, disrupted everything else, leaving a random coil. The amino acids were still in their proper sequence, but the enzyme was no longer active. He then found that if he removed the mercaptoethanol, he could regenerate cross-links between the cysteines—but the links were random, not the four unique ones that were found in the proper structure, and the protein did not regain its activity. However, if he simultaneously removed the mercaptoethanol and urea slowly, the protein would reform its native structure, and its original enzymatic activity would be regenerated.

You really need to think in terms of a landscape in which high-energy conformations are hills, low-energy ones are valleys, and the conformation of the protein at any given moment is tracked by a boulder that always wants to roll downhill.

This showed that the native fold must be the most stable form thermodynamically. Anfinsen didn't add any energy-producing molecules, or any chaperones. The molecule found the correct structure on its own, so that structure must be the most stable configuration under physiological conditions.

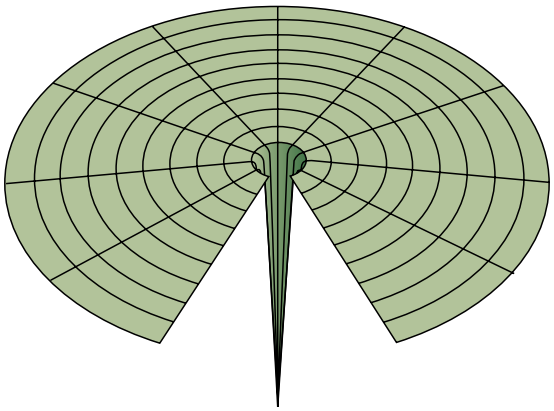
So, how *do* you get from a protein in total disorder to this end point? In the mid '60s, Cyrus Levinthal, then at MIT, proposed a thought experiment. Assume you have a protein with 100 amino acids in it. That's small, but a reasonable starting point. And say that each amino acid can assume just two conformations, a vastly simplifying assumption. We know from physical-chemistry experiments that these conformations interconvert on the scale of a picosecond, or 10^{-12} seconds. So two conformations per amino acid times 100 amino acids gives 2^{100}, or approximately 10^{30}, total conformations, and if it takes a picosecond to make each change, that suggests that the time to sample all possible conformations will be 10^{18} seconds, or some 10 billion years. The problem with that is that the age of the universe is only about 12 billion years, so this can't be the way to fold proteins.

Levinthal knew this was a straw man. Instead of talking about conformations and interconversion rates, you really need to think in terms of a landscape in which high-energy conformations are hills, low-energy ones are valleys, and the conformation of the protein at any given moment is tracked by a boulder that always wants to roll downhill. Levinthal assumed that all the "wrong" conformations were equally probable, which meant that they all had the same energy. In that case, the landscape would look like a putting green—a very small hole somewhere on a huge, flat surface. The chances of the ball dropping into the cup just by rolling at random over the green are exceedingly small, and that's why it would take forever to fold the protein.

More recent theoretical work by a number of people, including José Onuchic (PhD '87) and Peter Wolynes at UC San Diego, suggests that the energy landscape is more like a funnel. For a lot of the really extended, unfolded conformations, rotating part of the molecule around one bond doesn't change the energy very much. So those conformations are equally probable, and the surface way out there is pretty flat. But as you start forming one or two of the weak interactions that are present in the native structure, you stabilize that conformation a little bit. This stability lowers its energy, and that puts you on the lip of the funnel. From there, you can follow a trajectory that is much faster than

If all the wrongly folded conformations had the same stability, the energy landscape would look like a putting green (right). But the three-dimensional structure gets more stable as various parts of it find the correct conformation, making the surface look more like a funnel (far right).

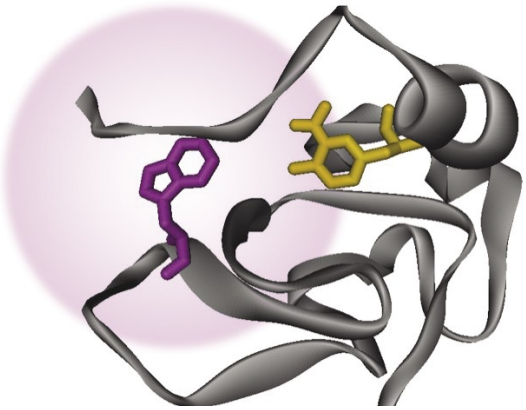
Adapted from Dill and Chan, "From Levinthal to Pathways to Funnels," *Nature Structural Biology*, Volume 4, No. 1, January 1997.



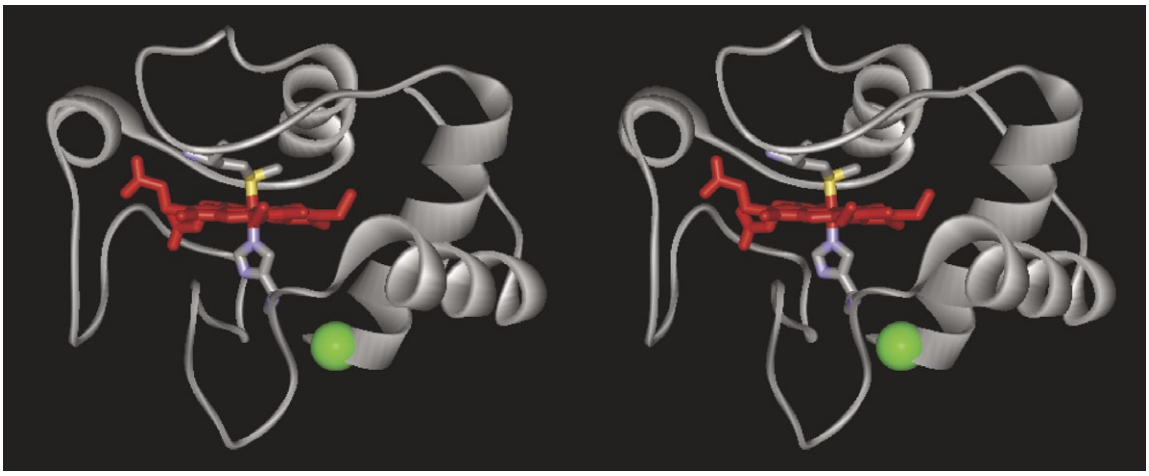
randomly searching every different conformation. Moreover, you don't have to follow a single pathway. There are many possible routes downhill—you don't need one unique set of events to occur in the proper order for each and every molecule. Mind you, there are still ways to go wrong; there are little traps near the bottom of the funnel, local minima, where you could get stuck. So you may have to do some corrections, but you've solved the big problem—once the slope starts to drive you toward the native structure, you need only search through a relatively limited number of configurations.

Here at the laser lab, we decided to try to develop a method for watching the protein as it's folding. All the structures I've shown you were determined by X-ray crystallography, which requires that you prepare a single crystal of the protein—a regular, repeating lattice of protein molecules—which is a notoriously difficult feat, even for a properly folded protein. You then shoot X-rays at the crystal, which diffracts them at various angles and intensities, and by working backward from the diffraction pattern you can deduce the arrangement of atoms that produced it. But a moving, refolding protein doesn't have a regular, repeating lattice. So we use a spectroscopic technique called fluorescence energy transfer, which tells us about the distance between two amino acids of our choice. There may be hundreds of amino acids in the protein and we can only look at two of them at a time, so we don't get anywhere near the amount of information that we do from X-ray crystallography. But if we look at enough different pairings, we can start putting together a picture of what the disordered protein looks like and how it reorders itself as it folds up.

To do energy transfer, we need an energy donor and an energy acceptor. The amino acid tryptophan makes a good donor—when excited with ultraviolet light, one of its electrons jumps to a higher energy state. Within about 10 nanoseconds, or billionths of a second, the molecule reradiates that energy, or fluoresces, at a slightly different wavelength. So if we have an acceptor molecule such as nitrotyrosine, a slightly modified amino acid that has an excited state at a similar energy, the reradiated energy can be transferred to the nitrotyrosine. The rate of energy transfer varies as one over the sixth power of the distance, so if we can measure how fast the energy is transferred from donor to acceptor, we can calculate how far apart they are. We use a fast light detector called a streak camera to measure how the tryptophan's fluorescence decays with time. And we can use molecules in solution—we don't need crystals. Even better, we can collect a sequence of measurements on the

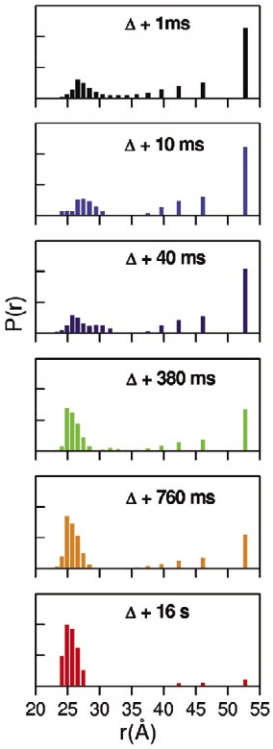


In fluorescence energy transfer, a donor molecule such as tryptophan (purple) radiates energy that is absorbed by an acceptor molecule such as nitrotyrosine (yellow). By measuring how fast this happens, you can tell how far apart they are. Here, the donor and acceptor are attached to a generic protein molecule.



Above: This stereoscopic image shows the three-dimensional structure of cytochrome C. To get the 3-D effect, hold the page about six inches in front of your face, so that one image fills the visual field of each eye. Relax and let your eyes cross slightly, and the 3-D image should pop into view. Cytochrome C has four α-helices. The heme acceptor sits in the center of the molecule and is colored red. The green sphere is the dye-labeled cysteine that acts as the donor.

Below: In this set of plots of cytochrome C refolding itself, r(Å) is the distance between the donor and the acceptor in Ångstroms, or ten-billionths of a meter. (Most atoms are a couple of Ångstroms in diameter.) The probability of finding the donor-acceptor pair at any given distance is P(r). The topmost plot is one millisecond (thousandth of a second) after the denaturant is removed; the bottom plot is 16 seconds after.



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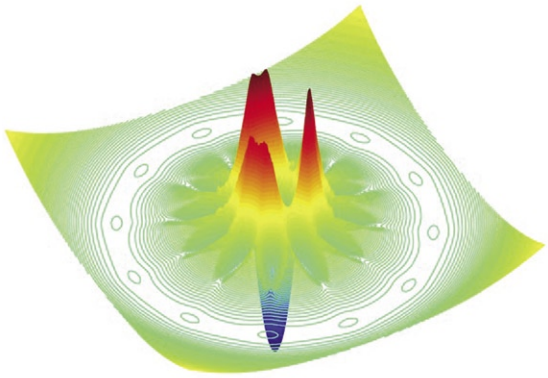
same molecules over time. And the best part is that we can use standard molecular-biology techniques to insert our donor and acceptor anywhere we please—with some caveats—in the amino-acid chain.

Now, a nicely folded protein with a single distance between the donor and acceptor would give a single energy-transfer rate. But a disordered protein has a whole distribution of distances, and we can watch how the distribution changes as the protein refolds. Julia Lyubovitsky (PhD '03) first did this with a protein called cytochrome C, but she didn't use nitrotyrosine and tryptophan. For the energy acceptor she used a part of the protein called a heme, which is very much like the iron-containing molecule in hemoglobin. The heme is bound to the protein by a histidine at position 18. She then reacted the cysteine at position 102 with a dye molecule that acts as the donor. (Remember, the number refers to the amino acid's position counting from the N-terminus.)

First, Julia unfolded the protein by adding guanidine hydrochloride, a denaturant similar to urea, to disrupt the three-dimensional structure. Then she did a set of initial measurements of the donor-acceptor distance distribution. Next, she quickly removed the denaturant in a fast-mixing experiment—basically, diluting it away by adding a buffer solution—and measured the distance distribution as it changed over time. Cytochrome C takes several seconds to completely refold, although some early events happen in tens of milliseconds. A time-lapse plot of the probability of finding a given donor-acceptor distance shows that there's a broad distribution of distances initially, and that the mean distance is relatively large. The heme and the cysteine are 84 amino acids apart, counting along the backbone, so if the protein is completely unfolded they can get pretty far from each other. But the mean distance gets shorter and the distribution of distances tightens up as the protein finds its way to the correct fold. At the end, you have a nice, narrow peak, with just a few molecules that didn't get it right.

Julia used this information to map the energy surface, and discovered that lots of extended, long-



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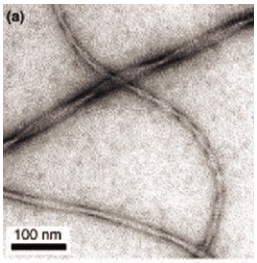
Cytochrome C's energy landscape has a broad, flat plain ringing the outskirts where many different conformations can interconvert freely. The deep funnel (blue) in the middle has the correct fold at its bottom. It is guarded by a ring of mountains, but the passes between them aren't very high and are easily traversed with the energy available to the protein at room temperature. The box canyons on the mountains' flanks are topologically frustrated energy traps, but they're shallow and easy to get out of unassisted.

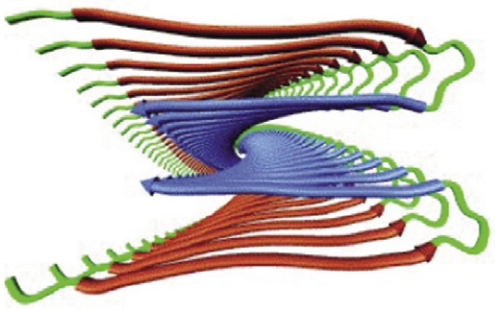
distance structures remained throughout the course of refolding. In other words, once she removed the denaturant, the protein didn't first wad itself up and then wriggle around to try to find the native structure. Instead, about half the molecules stayed well extended while the others collapsed down. Interchange between the extended and collapsed conformations proceeded on time scales of roughly a hundred microseconds. The nearly equal populations of the two conformations indicate that the extended structures lying out toward the edge of the energy surface are not substantially less stable than the collapsed structures.

And this is good, if you think about it, because very often you can get a nonproductive collapse. If the protein winds up with one part of its chain on the wrong side of another, you're stuck. The chains can't pass through one another, so you'd have to break one in order to get to the correct fold. This is called topological frustration. If these wrong structures are really stable and have deep energy traps, you'll have a lot of problems trying to fold the protein. But if these topologically frustrated structures tend to unfold, the protein can go back out to the rim, race around a bit, and hope to recollapse on a more productive route. If the traps aren't very deep, there are many chances to unfold and try again. We think this is an important insight into how proteins avoid getting misfolded. Postdoc Kate Pletneva is developing a more detailed picture of the cytochrome C folding landscape, using six different versions of the dye-labeled protein.

So far I've talked about how things go right, but diseases happen when things go wrong. A protein could misfold because of a mutation in its amino acid sequence, or environmental stresses might lead it to partially unfold and then set it on a misfolding path.



Above: An electron microscope image of Alzheimer's-type amyloid fibrils, with the twist clearly visible. The scale bar is 100 nanometers, or billionths of a meter, and the fibrils are typically 0.1 to 10 microns (millionths of a meter) long. Above, right: A schematic view of the twist, in which each blue arrow represents a β-sheet. From R. Tycko, *Current Opinion in Structural Biology*, 14, 96-103 © 2004, with permission from Elsevier.

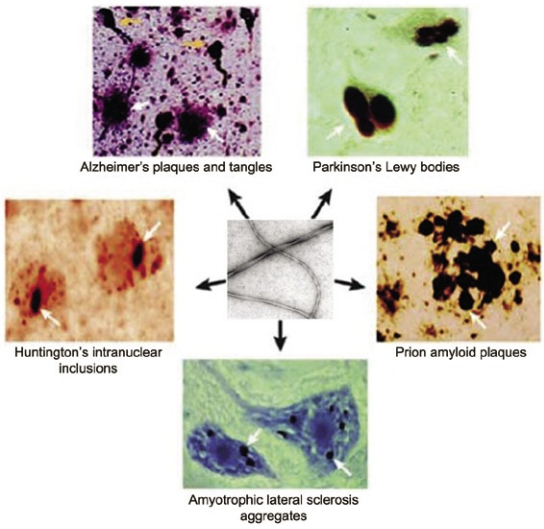


R. Tycko, *Current Opinion in Structural Biology*, 14, 96-103 © 2004, with permission from Elsevier.

Each β-sheet consists of two amyloid molecules, each of which in turn forms two β-ribbons. The four-stranded β-sheets then stack as shown here (for clarity, only the amino acids at positions 9-40 have been included), with the fibril's long axis coming out of the page toward you.

There's a large and growing list of neurodegenerative diseases that are characterized by insoluble deposits of misfolded proteins. With a few exceptions, proteins that work inside a cell need to be soluble, but the deposits are basically rock-solid masses—tangled, insoluble fibrils of the misfolded protein that trap a bunch of other stuff in with them. Besides Parkinson's disease, brain-tissue samples from Alzheimer's disease; Huntington's disease; amyotrophic lateral sclerosis, better known as ALS or Lou Gehrig's disease; and prion diseases all show fibrils that look pretty much the same, but in each case it's a different protein. In Parkinson's disease, the protein is α-synuclein. In Alzheimer's, it's a relatively short β-amyloid peptide of 42 amino acids. In Huntington's, it's a protein called huntingtin. In ALS, superoxide dismutase is involved. And in prion diseases—which include mad cow and its human analog, Creutzfeldt-Jakob disease or CJD—it's the prions, which are infectious protein particles. In each case, the details are different. In some diseases, the masses form between cells. In others they form within cells. They look somewhat different, and have different names—in Parkinson's, for example, they're called Lewy bodies; in Alzheimer's they're called plaques.

Regardless of what the protein is, all of these misfolds have a similar structure in which the strands, instead of forming helices or whatever, lay out in β-sheets. The sheets lie parallel, stacked perpendicularly to the fibril's long axis, with a little bit of a twist as you go along the fibril. But the sequence of amino acids determines the proper fold, so how can this be? Well, a few years ago Chris Dobson at Cambridge University found that by using the right solvent and temperature conditions, he could pick just about any protein and induce it to form this structure. All the sequence information seems to become unimportant, because these structures



Postmortem brain-tissue samples from several different diseases show dark masses (white arrows) of misfolded proteins. From Claudio Soto, *Nature Reviews Neuroscience*, 2003, 4, 49–60, with permission from Nature Publishing Group.

arise not from the side chains of the amino acids, but from their backbone, which is the same for all of them. Anfinsen's experiment said that the properly folded structure was the most stable, but these aggregates can be even more stable because they're insoluble. It's a one-way ticket.

We've been studying α-synuclein, which is associated with Parkinson's disease. According to an article in the July 2005 issue of *Scientific American* ["New Movement in Parkinson's" by Andres Lozano and Suneil Kalia], Parkinson's afflicts at least four million people worldwide, including as many as one million Americans, and about half of its victims begin to display symptoms before age 60. Parkinson's disease results from the loss of nerve cells, or neurons, in a small part of the brain called the substantia nigra. These cells produce dopamine, a neurotransmitter associated with movement. As they die, your dopamine level drops, and that leads to the tremors, dyskinesia (jerky, uncontrollable twisting or flailing motions of the limbs), and "freezing" that are characteristic of the disease. There is no known way to prevent Parkinson's, or stop (or even slow) its progression, but its symptoms can be greatly reduced by drugs and other therapies, including the implantation of a pacemaker-like device that delivers electrical stimulation to cells deep in the brain that control movement.

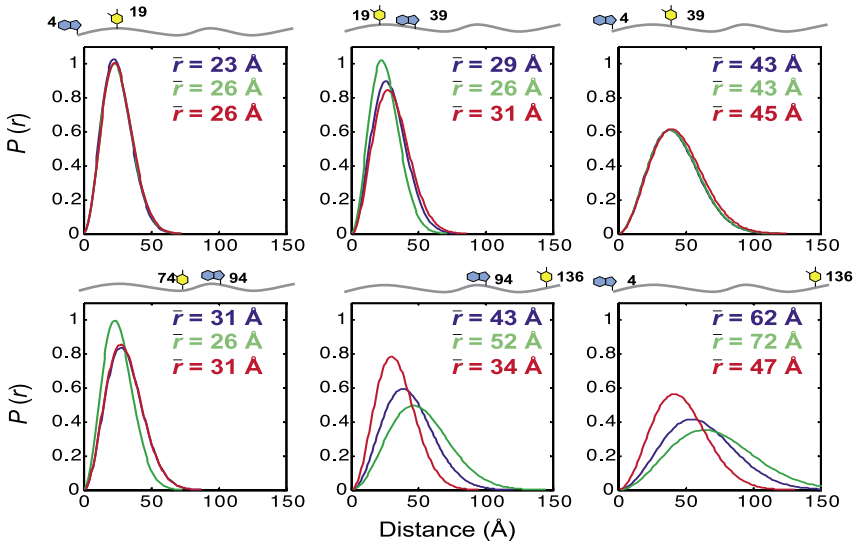
IMAGE NOT AVAILABLE

The substantia nigra is a small region deep in the brain. Named for its black color, the region contains dopamine-producing neurons. As these neurons die, the color fades.

Interestingly, there's a synthetic analog of heroin called China White that, within days of use, sometimes induces irreversible symptoms that are virtually identical to Parkinson's, including the development of Lewy bodies. J. William Langston of the Parkinson's Institute in Sunnyvale, California, studied these so-called "frozen addicts" in the early '80s, and discovered that the culprit was an impurity called MPTP, for 1-methyl-4-phenyl-1, 2, 5, 6-tetrahydropyridine. MPTP is now used in laboratory studies on mice and rats, which don't normally develop Parkinson's disease—possibly because they don't live long enough. But you give them MPTP, and they accumulate α-synuclein deposits, and in some cases develop symptoms resembling Parkinson's.

As I said, the Lewy bodies are primarily composed of α-synuclein. Alpha-synuclein is a small protein, only 140 amino acids long, and is widely found throughout normal brain tissue. Its function is not yet known, but the speculation includes such divergent roles as helping the right synapses form during learning, aiding in membrane formation, and moving fatty molecules called lipids around. It's found in the cytosol, the liquid inside the neuron, but it's also associated with the membranes of synaptic vesicles, which are the sacks that store neurotransmitters.

Oddly, α-synuclein doesn't appear to have a well-defined structure. If you dissolve it in a solution containing membranes or membrane mimics, it'll cling to them and start to form some α-helices, but it never assumes a single, discrete conformation. But if you take a solution of α-synuclein and let it sit at 37 degrees Celsius or so for several days, it will form fibril deposits completely on its own, in



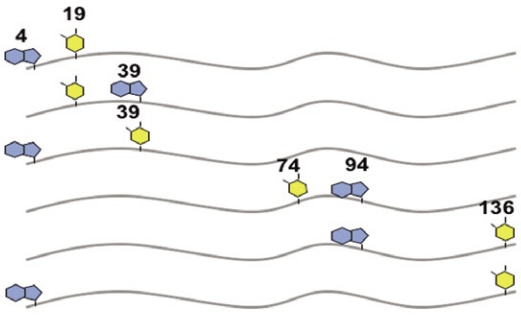
The data for each donor-acceptor pair shows the probability of finding a given donor-acceptor distance *P*(*r*) versus the distance in Ångstroms under three sets of conditions. The blue is normal physiological conditions, the green is after adding a membrane mimic, and the red is after adding acid. The colored numbers give the mean donor-acceptor distance in each case.

the absence of any cellular machinery.

Because of this lack of structure, we thought α-synuclein would be an ideal candidate for our fluorescence energy-transfer methods. Beckman Senior Research Fellow Jennifer Lee (PhD '02) used the tryptophan donor and the nitrotyrosine acceptor, placing donor-acceptor pairs in the protein's N-terminal region, the central region, and the C-terminal region, as shown below. She also put the donor at the N-terminus and the acceptor at the C-terminus to see how close the two ends got to each other. And she made two more pairs by putting the donor and acceptor, both of which have big, bulky ring systems, at various spots where there already were big, bulky ring systems, on the logic that this would cause the least distortion in the structure. Then she measured the energy-transfer kinetics for each of the six pairs, and mapped out the distributions of donor-acceptor distances.

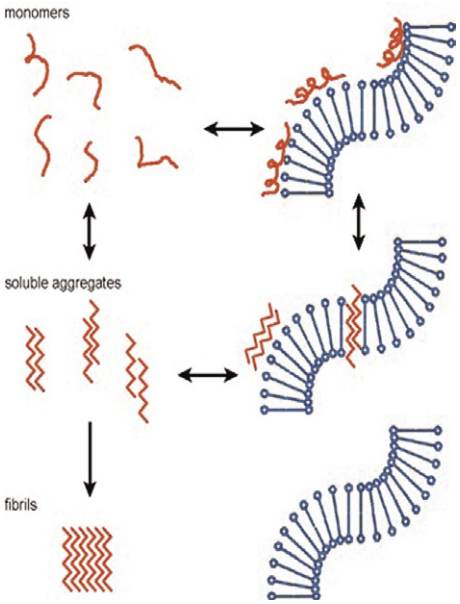
The donor-acceptor distance-distribution curves under various conditions are shown above. By themselves they don't offer much information, but they do give us some constraints. We're working with Vijay Pande, a computational chemist up at Stanford, to plug these distributions into his molecular-dynamics software to try to get a feel for the families of structures that may exist in solution. The blue curves in the figure are for the molecule at the level of acidity found in our cells, that is, at pH 7.4. I'll get back to the green and red curves momentarily.

It's known that α-synuclein associates with membranes, and it's been suggested by, among others, USC's Ralf Langen (PhD '95) that the α-helices I mentioned earlier allow the protein to lie down on the membrane's surface. But as the protein molecules start to aggregate into twos and threes, the helices uncoil and the protein's structure becomes more like β-ribbons. This has caused some people, notably Peter Lansbury at Harvard and Brigham



Six different versions of α-synuclein were made, each with the donor (blue) and acceptor (yellow) at different sites.

Right, top: The helices of individual α-synuclein molecules (red) may allow the molecules to adhere to membrane surfaces (blue). Middle: But as the molecules start to aggregate in twos and threes, they may begin to form β-ribbons that may penetrate the membrane, causing the cell to spring a leak. Bottom: The ribbons eventually form fibrils, keeping them from doing further damage.



1 10 20 30 40 50 60
MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGG

The amino-acid sequence for α-synuclein, using the one-letter codes shown on page 14. The two underlined regions form the two helices, and are largely made up of an almost-identical repeating unit shown in red. (After T. S. Ulmer *et al.*, *Journal of Biological Chemistry*, 2005.)

and Women's Hospital, to think that the β structures form a pore, possibly like the β-barrel I described earlier, that penetrates the membrane and leads to leakage and eventually cell death. In that case, forming the insoluble fibrils may actually be a protective mechanism. This really points out how little we know—even though the fibrils are a hallmark of the disease, they may not be the problem. It could be that their precursors are really what's killing the cell, and the fibrils are the cell's attempt at self-defense. It's hard to find out what's really going on, because working with cultures of nerve cells is a very tricky business. You just look at them cross-eyed and they'll die on you.

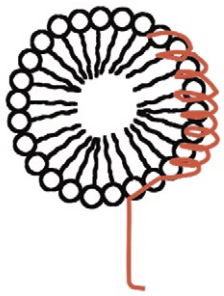
NMR data suggest that one membrane-bound structure may have two α-helices, with a small flexible region in between. (Whether these helices actually lie flat along the membrane or are embedded into it isn't known.) The green curves in Jennifer's data show what happened when she added sodium dodecyl sulfate (SDS) micelles, a membrane analog, to the solution. A molecule of SDS, known to the nonchemist as "soap," has a negatively charged head and a long, oily tail. Above a certain concentration, the molecules form little spheres, called micelles, with all the heads on the outside and the tails in the interior. Micelles make good stand-ins for biological membranes. Jennifer found that the N-terminal region stayed the same or even shortened up a bit, perhaps showing more helical character; while the C-terminal region seemed to stretch out. This is probably an electrostatic effect, as the outside surface of the micelle and the C-terminus of the protein both have negative charges that would tend to repel each other.

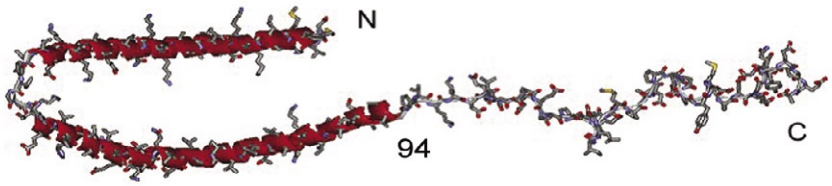
We don't really know that the amino-acid backbone bends to put the two helices side by side, the way they've been drawn, but we plan to find out. Jennifer is going to put a donor at the N-terminus and an acceptor at the C-end of the second helix, at roughly position 94. If this partially straightened paper-clip-like structure is correct, we should see a short distance. NMR tends to show the most stable structures, but it also points to places where we should put donors and acceptors. So combining these techniques is potentially very useful.

It's been found that the insoluble aggregates form faster under acidic conditions, so Jennifer also did a set of studies at pH 4.4. The red lines in the plot on the opposite page show that there's not too much change until the C-terminal region, where you see much shorter distances. This shortening, again, is probably primarily electrostatic in nature. As we acidify the solution, the negatively charged and weakly acidic carboxylic acid group on the C-terminus accepts a positively charged hydrogen ion from the solution and becomes electrically neutral. The C-termini are now more inclined to snuggle up, rather than being repelled by one another. We don't yet understand how the conformations we find in solution relate to the propensity for fibril formation—as you can see, the relationship is not straightforward. (Jennifer's samples did not make fibrils, as she was working at low protein concentrations where they don't form.)

While Parkinson's is usually caused by a mix of genetic and environmental factors, about 5 percent of the cases are strictly genetic, says *Scientific American*. There are several different mutations that can cause Parkinson's, at least two of which occur in α-synuclein. If your DNA replaces the amino acid named alanine at position 30 with a proline, or the alanine at position 53 with threonine, you will develop the disease while you're still in your 30s. Jennifer looked at how the shape of

An α-synuclein molecule can coil up and lie down on an SDS micelle the way it does on a membrane.





This structure of α-synuclein bound to a membrane mimic was determined by NMR spectroscopy. (After T. S. Ulmer *et al.*, *Journal of Biological Chemistry*, 2005.)

70 80 90 100 110 120 130 140
AVVTGVTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNEAYEMPSEEGYQDYEPEA

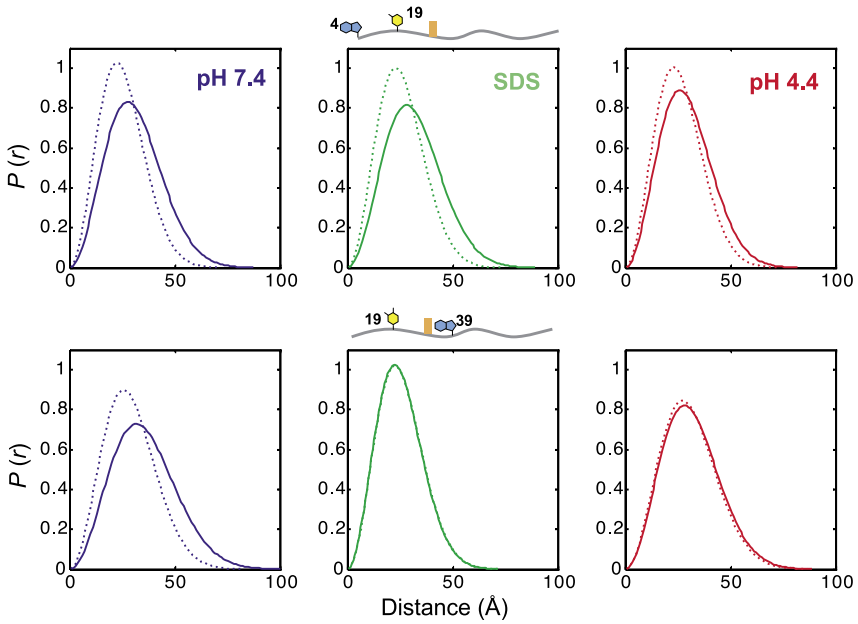
the molecule changed when she made the alanine-proline mutation, placing a donor-acceptor pair on the N-terminal side of the mutation, and a donor-acceptor pair to span the mutation site. She looked at the distance distribution at physiological pH, in the presence of SDS micelles, and under acid conditions. In all three cases, she found elongation at the N-terminal region when she introduced the mutation. Interestingly, however, when the donor-acceptor pair spans the mutation, you only see significant lengthening at normal pH, pH 7.4; you don't see a substantial change in structure in the presence of SDS micelles or at acid pH. We still don't understand the molecular basis for this change. We do know that it's not electrostatic, but we need more data to figure out what's going on.

We're now bringing fluorescence energy transfer to bear on the aggregation of α-synuclein into fibrils. I mentioned that the toxic form may actually be the fibrils' soluble precursors, and we think our method will give us some insights into them. We'll put a small concentration of our protein in a solution of the regular protein, and we hope to see structural changes as the protein starts to aggregate before the solids start forming.

While we've been concentrating on α-synuclein, and we hope to make a contribution to untangling the role that misfolded proteins play in these debilitating neurological diseases, you can see that fluorescence energy transfer is a very general technique. You can look at protein structures in solution, and you can follow what happens when the protein interacts with other molecules—signaling molecules, drugs, environmental agents—under various conditions. It's really a very basic, powerful tool for molecular biology, with applications to essentially any protein system or cellular process. □

Jay Winkler received a BS in chemistry from Stanford University in 1978. He received his PhD in chemistry from Caltech in 1983, working with Beckman Professor of Chemistry Harry B. Gray on, among other things, electron transfer in ruthenium-modified cytochrome C. After a two-year postdoc with Norman Sutin and Tom Netzel at Brookhaven National Laboratory, he received an appointment as a staff scientist there and resumed studying electron-transfer reactions in various proteins. In 1990, he returned to Caltech as a Member of the Beckman Institute and Director of the Beckman Institute Laser Resource Center.

PICTURE CREDITS:
14, 16, 17 – Jay Winkler, Doug Cummings; 17, 20, 21, 22 – Jennifer Lee



Swapping the alanine at position 30 (orange block) with a proline causes early-onset Parkinson's. Shown here are distance-distribution data from the N-terminus (top row) and spanning the mutation site (bottom row). The dotted line is for normal α-synuclein, and the solid line is the mutant version.