made repeated measurements to monitor the characteristic time over which the spin-state oscillations decayed. That work now sets the stage for statistical studies that explore the link between an NV center's decoherence and local magnetic fluctuations in processes such as the charge transport through ion channels in a cell membrane. Last year Hollenberg and colleagues calculated that ion-channel dynamics could, in principle, be detected with millisecond resolution by monitoring the probe's decoherence.[4] The issue is not just academic; ion channels are important drug targets. **Mark Wilson**

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Kinetic experiments shed light on protein-folding thermodynamics

Perturbing biomolecules and then watching them relax may be the kind and gentle way to determine their free-energy landscapes.

In its native state, the treasure trove of nutrients and biochemical machinery known as egg white is a slimy, translucent soup of proteins. Heat it atop a stove, however, and the proteins unfold, coagulate, and collectively morph into an opaque white solid. The unraveled proteins are said to have denatured. The enzymes among them, although well-suited for a cheese omelet, are in no shape to usher along biochemical reactions.

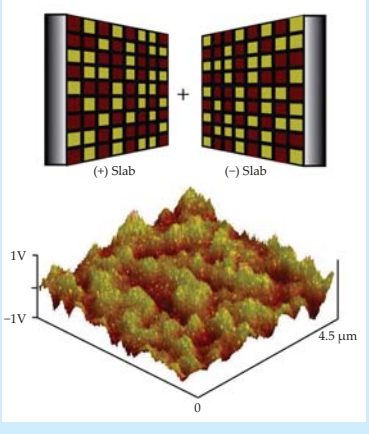
The proteins would have suffered a similar fate had the egg white been whipped into a foamy meringue or soaked in lime juice. Indeed, the precise biological work of folding a protein can be undone by any number of environmental stresses, including heat, acidity, and mechanical strain. Proteins, like all molecules, tend to adopt the shape that minimizes their free energy. In some circumstances, a compactly folded state makes thermodynamic sense; in others, it doesn't.

To characterize the influence of environment on a protein's shape, biologists construct a free-energy landscape. They typically do so by performing titrations, experiments not all that dissimilar to frying an egg: A protein solution is subjected to a gradual ramp in some input variable—perhaps temperature, perhaps some other quantity—and monitored for physical changes indicative of folding or unfolding.

physics update

These items, with supplementary material, first appeared at http://www.physicstoday.org.

A nanoscale mosaic model of static electricity. Rub a balloon against your hair or rub any two nonconducting materials together, and as any high school physics student knows, the surfaces develop opposing static charges. Theoretical models of that process, known formally as contact electrification, have long assumed that the material properties of each surface are spatially homogeneous and that the post-contact charge distributions are uniform. If those assumptions were correct, then identical materials rubbed together should not transfer any charge. But they do, as was demonstrated by Northwestern University researcher Bartosz Grzybowski and colleagues roughly two years ago with identical polymer slabs. They predicted then that charge is transferred through a random mosaic of oppositely charged submicron-scale domains, shown in the schematic, generated by inhomogeneities in the materials' surface properties. Now, Grzybowski and other Northwestern researchers have experimentally verified the mosaic model by imaging contact-electrified polymer slabs with an atomic force microscope; the AFM-generated surface potential map shown here revealed multiple positively and negatively charged nanometer-sized domains. Probing further, the researchers found possible evidence of surface inhomogeneities: Raman spectra indicated that some bonds were cleaved or oxidized, and x-ray photoelectron spectra of dissimilar polymer slabs that had been in contact revealed nonnative elemental peaks, which suggests material transfer. Their next goal is to probe other local surface properties to find out how bond breaking and material transfer influence domain size and overall charge. (H. T. Baytekin et al., *Science*, in press, doi:10.1126/science.1201512.) —JNAM



Tantalizing and rare neutrino oscillation. The first appearance of electron neutrinos amidst an underground beam of muon neutrinos has been reported by Japan's T2K collaboration. The three "flavors" of neutrinos—electron, muon, and tau—can quantum mechanically swap identities in transit as long as all three neutrino masses are different. To date, those so-called flavor oscillations have been detected mainly by observing the disappearance, rather than the appearance, of neutrinos of a given flavor; the assumption is that some of the missing neutrinos changed identity en route from their source. Originating at the Japan Proton Accelerator Research Complex (J-PARC), the T2K muon-neutrino beam traveled 295 km to Japan's Super-Kamiokande detector, where 88 neutrino-interaction events were detected. Of those 88 events, 6 appear to come from electron-type neutrinos. Only 1.5 such events would be expected if the elusive flavor-mixing parameter θ_{13} were zero. The θ_{13} result, based on only 2% of the data originally expected from the experiment, is considered preliminary. But it is being published because J-PARC was damaged by eastern Japan's massive earthquake and tsunami on 11 March 2011 and will remain offline for many more months. If confirmed, the result will have profound implications: A nonzero θ_{13} makes possible *CP* violation with leptons, which might then explain the cosmic matter–antimatter imbalance. (K. Abe et al.: T2K collaboration, http://arxiv.org/abs/1106.2822.) —SGB

Houston's structures thwart cleansing breezes. On 30 August 2000, as the Sun beat down on Texas's largest city, ozone

But as Martin Gruebele of the University of Illinois at Urbana-Champaign can attest, the titration approach has its limitations. Gruebele is one of a growing number of biophysicists who are interested in understanding how proteins fold inside living cells and organisms. He and his coworkers have learned firsthand that many cells, including the cancer cell shown in figure 1, can't survive the considerable knob-turning required to complete a titration.

Now a team led by Gruebele and Yann Chemla (also at the University of Illinois) has arrived at a potentially less destructive way to generate folding free-energy landscapes.[1] The researchers' theory, simulations, and experiments endorse a counterintuitive strategy: To get the clearest picture of a protein's folding equilibria, force it out of equilibrium.

The baseline problem

The dramatic change in texture and appearance that befalls a frying egg is only a crude and indirect indicator of protein denaturation. To precisely characterize folding transitions, biologists look for more subtle clues, such as a shift of a peak in the protein's vibrational spectrum or a change in the emission of a fluorescent probe.

Ideally, the observed property would produce an output signal B that changes only when a protein folds or unfolds. Plotted as a function of the input variable, B would describe a sigmoid curve. The two horizontal tails would correspond to folded and unfolded states. The transition point, at which half the proteins are folded and

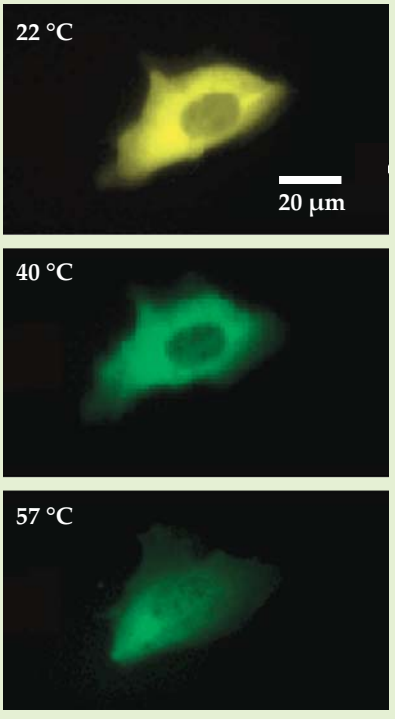
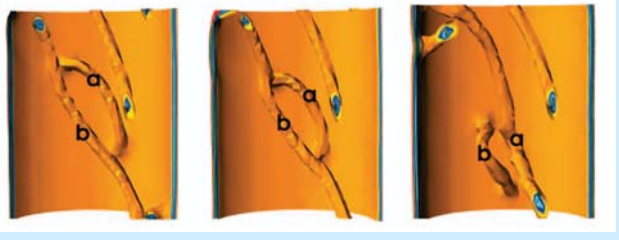


Figure 1. The structure of the fluorescent-tagged phosphoglycerate kinase enzymes in this bone-cancer cell can be inferred from their emission spectrum. The cell's yellow appearance at 22 °C indicates the enzymes are in their native, folded state. The green emission at 40 °C signals that several of the enzymes have unfolded. But at 57 °C, a measurement point needed to round out the titration data, the cell has already died. (Images courtesy of Martin Gruebele.)

concentrations soared to unhealthy levels. Usually in summer, as the city heats up, sea breezes blowing in from nearby Galveston Bay and the Gulf of Mexico refresh the air. But the prevailing winds over Houston, although mild, tend to counteract the sea breeze. Thus, if the breeze collides with the prevailing winds, stagnation sets in over the city and pollutants can build up. Now a numerical study led by Fei Chen of the National Center for Atmospheric Research suggests that the materials of the urban environment are partly to blame for ozone pollution. Chen and colleagues validated their computer model by comparing their simulation of the August 2000 pollution event against extensive data collected in the Texas Air Quality Study 2000. Then, to understand how various environmental features affect the development of the sea breeze, they simulated conditions that were wetter or dryer than normal, and in one simulation they replaced the urban landscape with cropland. The substitution of crops for concrete had the greatest impact on boosting the sea breeze and reducing periods of stagnation. (It also increased the efficacy of the nighttime land breeze that blows pollutants out to sea.) Compared with green space, the researchers found, the urban environment is hotter. That effect actually tends to enhance the sea breeze, but the enhancement is more than offset by the frictional damping from Houston's buildings. (F. Chen et al., *J. Geophys. Res. [Atmospheres]*, in press, doi:10.1029/2010JD015533.) —SKB

Stirring superfluids. If you chill fermions enough, they can pair up to form bosons and settle into a single collective ground state, a Bose–Einstein condensate. In the case of helium-3 atoms, the resulting BEC is a superfluid that flows without dissipation—provided the flow is not so energetic that it breaks the pairs apart or destroys the ground state's coherence. Until now, theorists could characterize placid flows in fermionic superfluids, but not the vigorous turbulence that results from shaking or stirring. Aurel Bulgac of the University of Washington in Seattle and his colleagues have adapted density functional theory—a computational approach originally devised to calculate molecular energy levels—and applied its time-dependent extension to model turbulent fermionic superfluids. Although the underlying quantum mechanical equations are straightforward, solving them required the use of one of the world's most powerful supercomputers, Jaguar at Oak Ridge National Laboratory in Tennessee. In their simulations, Bulgac and his colleagues agitated a fermionic superfluid by shooting spherical projectiles through it or by stirring it with a laser beam. Turbulent superfluids are known to harbor tubes of quantized vorticity. As the figure shows, the simulation could track how two vortex tubes (marked a and b) joined to form a ring, which then opens in a manner reminiscent of the unzipping of a DNA molecule during transcription. Bulgac's model could help astronomers understand another agitated superfluid: the interior of a rapidly spinning neutron star. For more on quantum turbulence, see PHYSICS TODAY, April 2007, page 43. (A. Bulgac et al., *Science* **332**, 1288, 2011.) —CD



half are unfolded, would lie somewhere along the connecting curve.

In practice, factors other than folding and unfolding can influence how B changes with the input variable. An increase in temperature, for example, might alter an amino acid's vibrational frequencies, diminish the quantum yield of a fluorescent probe, or cause a folded protein to expand slightly without unfolding. Such events contribute to what's known as an intrinsic baseline. Because of them, the tails of real titration curves aren't horizontal but rather lie at slants, as illustrated in figure 2a.

To distinguish the folding transition from the intrinsic baseline, then, one needs to determine the slope of the upper and lower tails of the titration curve. That means acquiring data at values of the input variable ranging from well below to well above the transition point. For Gruebele's research group, it also meant that a tantalizing result lay just out of reach.

Thermodynamic nudges

Last year, Gruebele's group published experimental results showing that certain kinase enzymes can withstand higher temperatures inside a human cancer cell than they can in vitro.[2] It wasn't a complete surprise. Intracellular proteins are constantly jostling with other proteins, organelles, and cellular machinery; some theorists suspect that jostling could frustrate proteins' attempts to unfold. But the researchers couldn't obtain enough of the titration curve to fully pin down the enzyme's transition point. "We basically boiled our cells, and that was the end of the experiment," recalls Gruebele. "There had to be a kinder, gentler way."

Gruebele eventually came up with that kinder, gentler strategy: Give the biosample small, swift thermodynamic nudges. For many proteins, a step change in the input variable elicits a two-stage response like that illustrated in figure 2b. The nonfolding dynamics, the parts that make up the intrinsic baseline, equilibrate almost instantaneously, within picoseconds or nanoseconds of the step change. Folding transitions—much more complicated undertakings typically requiring a protein to climb a free-energy barrier—can take microseconds, seconds, or even hours.

As long as the slower folding dynamics can be sufficiently resolved, a protein's response to a step change can be separated into a fast part, ΔB_1, and a slow part, ΔB_2. The fast part is dispensable. The slow response, provided the step change is small, is proportional to the derivative of the titration curve, except

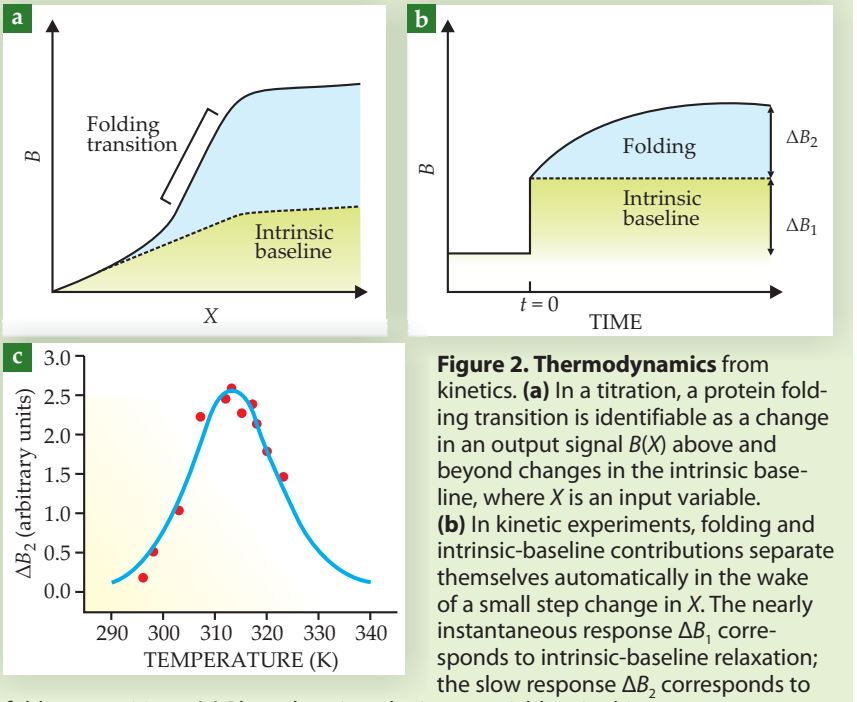


Figure 2. Thermodynamics from kinetics. **(a)** In a titration, a protein folding transition is identifiable as a change in an output signal $B(X)$ above and beyond changes in the intrinsic baseline, where X is an input variable. **(b)** In kinetic experiments, folding and intrinsic-baseline contributions separate themselves automatically in the wake of a small step change in X. The nearly instantaneous response ΔB_1 corresponds to intrinsic-baseline relaxation; the slow response ΔB_2 corresponds to folding transitions. **(c)** Plotted against the input variable—in this case temperature—ΔB_2 describes a bell curve, with most folding transitions occurring near the maximum. The data shown here are from in vitro experiments on SRC homology 3 proteins. (Adapted from ref. 1.)

with much of the unwanted intrinsic baseline conveniently discarded. Plotted against the input variable, ΔB_2 describes a bell curve like that shown in figure 2c, with the transition point lying near, if not precisely at, the maximum.

With the general concept in place, there remained some theoretical legwork. Technically, ΔB_2 corresponds to a change in some observable proxy, not a change in the folding-state population. (That's why the transition point doesn't necessarily coincide with the bell curve's maximum.) To connect those two dots requires a suitable model of the reaction kinetics and a handful of fitting parameters. Gruebele and company worked out the math for a two-state model.

There also remained the experimental legwork of demonstrating that the new approach, dubbed "thermodynamics from kinetics," actually works.

To the lab bench

The so-called SRC homology 3 domain (SH3), a sequence of roughly 80 amino acids that crops up in numerous proteins, is what Gruebele and his coworkers refer to as a "difficult case." SH3's structure can be probed using Förster (or fluorescence) resonance energy transfer (FRET) spectroscopy, a technique that reports the distance between two positions on a biomolecule's backbone (see PHYSICS TODAY, April 2011, page 16). But SH3 is so small—unfolded, its end-to-end span is a mere 30 Å or so—that folding-related changes in the FRET signal hardly stand out above the noise in the intrinsic baseline. SH3's temperature titration data resemble less a sigmoid than a steep, straight line.

To test the kinetic approach, Gruebele and company measured FRET signals of SH3 molecules responding to jumps of 2 °C; each jump was administered with a millisecond IR pulse. From the resulting data, obtained at the relatively mild temperatures of 45 °C and cooler, the team produced a more reliable estimate of the folding-transition point than they could have with titration data up to 59 °C.

The researchers chose SH3, however, specifically because it's ill-suited for titrations. Could there be an anti-SH3, some hypothetical combination of folding signal and intrinsic baseline that's more easily unscrambled by titration than by kinetics? "We tried to find one," Gruebele says, referring to a gauntlet of simulations the team conducted. "But even in the worst cases we could think of, our kinetic model did as well as the titration model. Most times it did better."

Ashley G. Smart

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