adding a humanistic touch to the mind-brain sciences. Feelings of love and loneliness, and the thoughts they provoke, are constructed in part from neural pathways in the brain that regulate core emotional responses, such as playfulness, sexuality, and friendship, as well as separation distress in our fellow creatures (*6*).

Of course, scientists should continue to be skeptical about such hypotheses until they are supported by solid research such as that carried out by Eisenberger and coworkers. Although there are many species differences in the emotional systems that we share as ancestral gifts with other animals (*6*, *22, 23*), the field of neuroscience will be more productive if it remains open to the similar nature of human and animal affective experiences.

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21. When I lost my daughter 12 years ago in a horrendous traffic accident, among her papers I found a poem that is now carved on her tombstone. The last stanza is particularly pertinent to the question of whether love can reduce the emotional pain of loss.

    When your days are full of pain,
    And you don't know what to do,
    Recall these words I tell you now
    —I will always care for you

    Full poem is published in A. Miller, *A Road Beyond Loss* (Memorial Foundation for Lost Children, Bowling Green, OH, 1995).
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CHEMISTRY

The Motions of an Enzyme Soloist

Michel Orrit

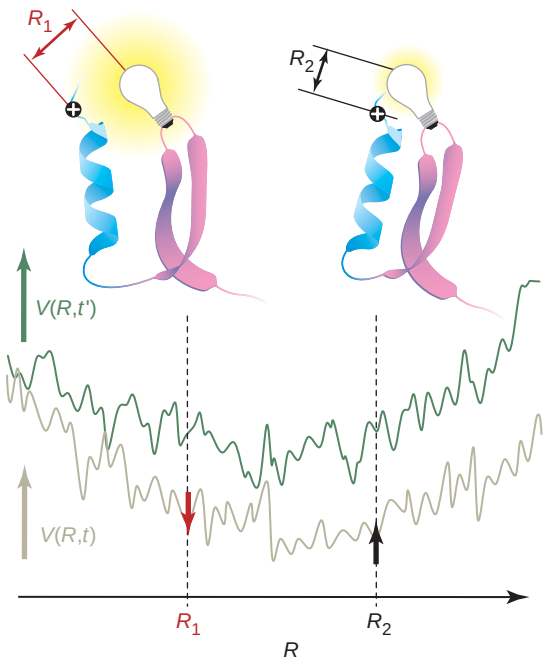
On page 262 of this issue, Yang *et al.* report a fluorescence study of the conformational fluctuations of a single enzyme molecule (*1*). As we can observe every time we boil an egg, proteins are not static. Conformational flexibility at various levels of their hierarchical structure enables proteins to fulfill a wide range of complex biological functions. Yang *et al.* now follow these fluctuations over a range of time scales at equilibrium, and not in response to some initial large disturbance.

Most proteins are highly complex macromolecules (*2*). Driven by Brownian motion, they wander in an intricate multidimensional energy landscape, featuring multitudes of interconnected wells and dells, valleys and passes. Hopping from well to well can be as fast as tens of femtoseconds for fast backbone vibrations, or as slow as hours and even days for folding and maturation in large proteins—a range of time scales spanning 18 orders of magnitude.

To make matters worse, protein functions such as catalytic reactions are determined by short-range atom-atom interactions and critically depend on tiny atomic displacements. Understanding of such subtlety and complexity is still in its infancy. Numerical simulations (*3*) can handle ever more complexity, but the longest simulations do not exceed a few nanoseconds. Moreover, computations must eventually be compared with experiments.

Current experiments to explore protein dynamics are of two kinds. First, fluctuation amplitudes may be measured with a range of techniques, each of which has a characteristic time window. The diffraction of x-rays and neutrons falls into this group, as do various spectroscopies, including nuclear or electronic magnetic resonance (*4*, *5*), Mössbauer absorption (*6*), infrared, and Raman spectroscopy. NMR is particularly powerful because it can distinguish each amino acid residue in the sequence, but it does not cover all fluctuation time scales.

Second, an ensemble of molecules may be synchronously brought out of equilibrium, and its subsequent relaxation monitored as a function of time. The initial disturbance in such kinetic measurements can be a temperature jump, a sudden concentration change, or—on much shorter time scales—the breaking of a bond by a laser pulse (*7*). Under such strong perturbations, however, proteins may no longer be close to equilibrium. Furthermore, synchronization is short-lived. Because different individual molecules follow different pathways in the energy landscape,



Monitoring dynamics via fluorescence. Variations of the distance R between an emitting fluorophore (flavin, sketched as a light bulb) and an electron acceptor and quencher (tyrosine, sketched as a black ball) cause variations of the fluorescence intensity and lifetime. The wiggly curve schematically represents a cut of the potential energy of the enzyme along R. Because the multidimensional potential depends on many other time-dependent atomic coordinates, the one-dimensional potential is time dependent and is shown here at two different times. The coexistence of low and high barriers in the transient potential generates stretched kinetics with a broad range of relaxation times.

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subsequent events occur over an ever wider range of delays.

Single-molecule methods (*8*) overcome the latter difficulty. Like an orchestra, a molecular ensemble needs to be synchronized for the outcome to be comprehensible. In contrast, a single molecule is a soloist, directly imparting its intricate improvisations to us. The pathway it takes can, in principle, be tracked as a function of time, allowing rare and potentially crucial events to be captured under physiological conditions and at equilibrium.

This dream has now come true for a single protein molecule (*1*). Yang *et al.* have followed the fluorescence lifetime of the flavin cofactor of a single enzyme as a function of time (see the figure). The flavin fluorescence is partly quenched by reversible electron transfer to a tyrosine residue. The transfer is sensitive to the distance R between flavin and tyrosine ($R \approx 0.45$ nm). Fluctuations of this distance by 0.1 nm cause variations of the fluorescence lifetime and intensity by a factor of 4.

These fluctuations lead to nonexponential fluorescence decay in an ensemble. Nonexponential decay is also observed for a single complex upon time averaging. The statistics of fluorescence lifetimes, or equivalently of distances R, relate to the thermodynamics of R, which cannot be distinguished from those of a harmonic oscillator at this stage. Much more interesting, however, are the correlation times of the fluctuations, which tell us how long a given conformation lives, rather than how often it occurs. The correlation times range from seconds to hundreds of microseconds (the latter limit being imposed by the weak fluorescence signal).

The broad distribution of the correlation times may not come as a surprise, but the quantitative fit of the correlation to a stretched exponential, and the absence of times longer than 1 s, are unexpected. Stretched kinetics are a signature of a broad distribution of relaxation rates, indicating that the potential has wrinkles and hills on scales finer than its overall harmonic shape.

Electron transfer probes donor-acceptor distances shorter than a few nanometers. It thus complements single-pair energy transfer (*9*), which is suited to longer distances (2 to 10 nm). The tyrosine-flavine distance monitored in the present work may or may not be relevant to the enzymatic reaction. More direct information on function may be obtained by labeling the active site. An even more ambitious goal will be to correlate enzyme-substrate conformations with activity fluctuations, which will have to be measured in real time.

Furthermore, deeper analysis of time trajectories of single molecules, such as that of Fig. 3C in (*1*), may reveal much more information than just a distribution of characteristic times. For example, the temporal order in which different conformations succeed one another may shed light on the topography of the energy landscape.

Relating function to structure and dynamics of proteins remains a major challenge in molecular biology. By adding a new method to our toolbox, Yang *et al.* have improved our odds for success.

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GENOMICS

Microarrays—Guilt by Association

John Quackenbush

From their inception, DNA microarrays (*1, 2*) have been touted as having the potential to shed light on cellular processes by identifying groups of genes that appear to be coexpressed (*3*). Although promising, this "guilt by association" approach has fallen into disfavor because of its many perceived limitations. On page 249 of this issue, Stuart *et al.* (*4*) weigh in with their study of the coexpression of groups of genes across species, which suggests that there may be merit to the guilt-by-association approach provided it is applied in an evolutionary context.

Most microarray experiments focus on identifying patterns of gene expression in a particular system—for example, comparing tumor tissue with normal tissue, analyzing gene expression responses to a stress stimulus, or comparing expression patterns in a particular tissue over time. Conceptually, differences between the various states should be reflected in changes in particular cellular pathways. Microarray data should allow users to identify the products of new genes and their contributions to these pathways. It is usually possible to identify cellular processes where most of the genes associated with a particular biological function are up- or down-regulated in a similar way. However, some genes known to be involved in a particular pathway invariably are missed, whereas other apparently unrelated genes exhibit expression profiles that are strikingly similar to bona fide pathway components. There is no consensus about how to interpret the gene expression patterns of hypothetical genes, genes of unknown function, or transcripts identified only by expressed sequence tags.

This perceived failure of microarrays has led some to portray the technique as "noisy" or "unreliable." Yet increasingly, when results from microarray studies are subjected to independent validation using other techniques (such as quantitative reverse transcription polymerase chain reaction), the confirmation rate—at least at the level of recapitulating the observed gene expression pattern if not the absolute magnitude—is well over 90%. So why aren't pathways emerging from microarray data that reveal groups of coexpressed genes?

One potential answer lies in the observation that many microarray studies fail to sufficiently sample the biological variability within a system. Increasingly persuasive arguments from statisticians (*5–7*), combined with improvements in the underlying protocols and technology used to collect expression data, have led to more sophisticated experimental designs encompassing increasingly broad surveys of diversity within a system. As a result, genes whose patterns of expression are identified as being statistically significant can be assigned a greater degree of confidence. Further, the validation rate—even among independent biological samples—is greater than in more naïve experimental designs. But has this resulted in the identification of new pathways? Not surprisingly, the answer is no. It seems that statistical significance is not always identical to biological significance.

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