Sheet." The "Fact Sheet" states that "DOE has no intention of becoming involved with setting the RERF scientific agenda." However, after a visit to RERF by DOE officials on 28 March that was intended to relieve our concerns, we continue to believe that the most fundamental issue has not changed and that DOE has indeed already had a substantial impact on the RERF agenda. We therefore continue to oppose the DOE proposal.

The basic issue involves the widely acknowledged need for a buffer between the DOE and RERF. This buffer must be capable of (i) protecting the credibility of RERF research in this controversial area; (ii) preventing even perceptions that the DOE can manipulate the scientific function of RERF to meet their own agenda; and (iii) ensuring the cooperation of the Japanese people, who have misgivings that the RERF project falls under the department that subsumed responsibilities for the development of nuclear weapons. There is no assurance that the DOE proposal can maintain these critical capabilities. Moreover, it would place responsibility for RERF policy and resources under direct control of laboratories and individuals conducting radiation effects research who would be competing for DOE support. This would create conflicts of interest by having the university group playing too many roles, at the same time managing and participating in RERF research efforts.

The DOE backed away from signing a previous arrangement with Columbia University, turning instead to a competitive process. They have recently notified several universities that a Request for Proposals will be announced in May. This would eliminate from consideration the NAS, which does not operate in the competitive arena. This is unfortunate because the NAS is uniquely situated to simultaneously meet important needs of this binational foundation and initiate needed research collaborations with U.S. universities.

The DOE has already exerted a strong influence over the RERF research program because their proposed change in contractor has imposed a hiring freeze that has prevented replacing crucial research staff. Moreover their entire approach to these issues has ignored the fact that there are binational mechanisms in place—a scientific council and board of directors—for consideration and direction of the RERF scientific agenda. We can see no reason why properly considered redirection of RERF efforts cannot be accomplished under the traditional NAS management, which has always involved universities and would continue to do so.

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Models of Protein Folding

Recent articles (*1*, *2*), including a Perspective by Peter G. Wolynes *et al.* about new insights into protein folding (17 Mar., p.

1619), have presented inferences derived from general thermodynamic or modeling perspectives, or both. These articles do not discuss in detail some of the best known and documented properties of native proteins. For example, native structures (not their enzymatic activity) are amazingly robust to mutations. Nearly any single residue may be replaced, one at a time, by any other with little or no detectable changes in the core secondary structures (*3*) or their relative orientations. In the extreme, all 100,000 or so sequence-distinct vertebrate globins, for example, have the same native fold. Even the Leghemoglobin, having no statistically significant sequence similarity, has the same basic fold. This is, of course, a requirement of a successfully evolving system. What does this say about the folding of native proteins? For one thing, they may not be typical of the vast majority of possible amino acid sequences, and their structures may be from a small and atypical region within the astronomically large conformational space available. Billions of years of selection can have profound and nonobvious effects. Thus there must be some question as to the validity of assuming that very general analyses or models, such as the various lattice models (*1, 4*), can be extrapolated to native proteins.

On the other hand, one of the ideas common to these recent discussions, the "funneled" energy surface, may be obvious by definition. That is, native proteins folding in aqueous solution at physiological temperatures do not get trapped in deep local minima. Native protein folding appears to proceed from a restricted conformational ensemble by condensation and secondary structure growth through an even smaller ensemble of "molten globules" to a thermally jittered final tightly packed "single" structure. Any polypeptide chain of near-native composition and length (80 to 300) will exist as a "loose globular state," or more correctly, as an ensemble of such states when placed in water. These ensembles, with the majority of the hydrophobic residues on the inside and the hydrophilics on the outside, represent some small fraction of the available in vacuo conformational space. Such aqueous ensembles will contain numerous secondary structure seeds composed of short helices and beta hairpins. These result from the burial of the backbone carboxyl and aminyl groups associated with the internalized hydrophobic residues and the enthalpic need to make up for the loss of their hydrogen bond interactions with the solvent. At physiological temperatures, different regions of these loosely folded structures will be continually sampling such seeds, no doubt in a highly parallel fashion. Any hydrogen-bonded structure that can "grow" and remain compatible with its neighbors in such loose structures will do so. Thus all native, and probably most random, peptide chains begin folding from these limited ensembles (the entropic width of the funnel's mouth) of loose globular hydrophobic cored structures containing many short transient hydrogen-bonded substructures and proceed down only those routes or "funnels" that are highly insensitive to sequence differences within the neighborhood of the native sequence or sequences.

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References

1. A. Sali, E. I. Shaknovich, M. Karplus, *Nature* **369**, 248 (1994).
2. D. Thirumalai and Z. Guo, *Biopolymers* **35**, 137 (1995).
3. R. B. Russell and G. J. Barton, *J. Mol. Biol.* **244**, 332 (1994).
4. E. I. Shaknovich, *Phys. Rev. Lett.* **72**, 3907 (1994).

Response: Smith raises an interesting set of questions about evolutionary selection and

the energy landscape viewpoint on protein folding [see reference (*1*) in our Perspective]. Smith's observations are in harmony with the energy landscape perspective on protein folding. The robustness of protein native structure to mutation is a consequence of the funneled nature of the energy landscape of a minimally frustrated protein. Kinetic ease of foldability, as we have argued, requires such a funneled landscape in which many of the interactions are consistent with each other. Modifying a few residues cannot dramatically change the final geometry at the bottom of a funnel. Frustrated heteropolymers, which are more common for completely random sequences, have multiple funnels, each leading to a different structure, and which configuration is the actual global minimum depends sensitively on the sequence, unlike natural proteins, which have been selected in evolution. The profound effect of years of selection is apparently to require proteins to obey the principle of minimal frustration.

Smith cites the example of Leghemoglobin, which has no statistically significant sequence similarity to vertebrate hemoglobin but which retains the same basic fold. Goldstein *et al.* have used the principle of minimal frustration to infer effective energy functions for protein structure recognition and prediction. The resulting algorithms do indeed recognize the structural similarity of the two globins (*2*). This shows the consistency and usefulness of the energy landscape analysis in understanding these distant relations.

Smith suggests that the funneled nature of the energy surface is obvious by definition. In fact, it is clearly not a definitional aspect of folding dynamics. Smith cites an experimental observation about proteins as the reason for funnels being "obvious." The question clearly answered by landscape analysis is "Why don't proteins get trapped in deep local minima while random heteropolymers typically would?" There is no longer any need to appeal to mysterious results of evolution.

Despite the naturalness of the qualitative funnel picture, the vast bulk of experimental work on the kinetics of protein folding has focused on the late-stage processes of slow-folding proteins, which are a bit more sequential and rather less parallel in character than the dynamics of the fast-folding stages. Energy landscape analysis explains how the discreteness of these late-stage processes, unlike the final structure itself, is a consequence of the ruggedness of the landscape that is sensed once a great deal of the folding has gone on. The energy landscape theory suggests these late-stage processes are sensitive to mutation and to details of the modeling. Experiment bears this out. In detailed study using nuclear magnetic resonance imaging, Dobson has shown that both hen egg-white lysozyme and human lysozyme, which share a common native structure, have different late-stage intermediates in folding (*3*). The energy landscape analysis suggests that the earlier and more important processes of self-organization are common to these sequences and follow the funneling mechanism restated by Smith. These events can be studied by simple models.

One significant aspect of the energy landscape analysis of protein folding is that it shows how one can reconcile different features of the mechanism of folding with the overall shape of a free energy surface. Equally important, however, is that it allows us to begin the quantitative analysis of folding dynamics. Using the energy landscape perspective to develop the mathematical correspondence between simple models and real proteins is an important step in moving from the qualitative philosophical discussions of protein folding mechanisms, which have been with us for many years, to a quantitative scientific understanding.

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References

1. J. D. Bryngelson *et al.*, *Proteins Struct. Funct. Genet.* **21**, 167 (1995).
2. R. Goldstein *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4918 (1992); *ibid.*, p. 9029.
3. S. D. Hooke, S. E. Radford, C. M. Dobson, *Biochemistry* **33**, 5867 (1994).

Corrections and Clarifications

In the news article "FDA puts the brakes on xenotransplants" by Rachel Nowak (5 May, p. 630) the description of the response of Columbia Presbyterian Medical Center in New York City to one of its investigators' proposal to test baboon-to-human heart transplants was incorrect. In fact, Columbia convened one panel of outside experts to advise its internal committees on the risks of xenotransplantation, and has since retained two outside experts as consultants to Robert Michler, the principle investigator for the xenotransplant trials. The institution also recommended that a group of experts on emerging infections be convened to examine the risk of viral transmission associated with xenotransplantation (Letters, 21 Apr., p. 349).