

sary and sufficient condition for a sequence to fold rapidly in the present model is that the native state is a pronounced energy minimum² and “the features that depend only on the lower discrete part of the spectrum can be characterized by use of the compact self-avoiding chains alone, neglecting the non-compact conformations”². But this conclusion is contradicted by 11 of the sequences they studied for which lowest-energy conformations are not maximally compact². The true denatured ensemble is generally much larger than the maximally compact ensemble, so neither the first excited state “energy gap” ΔE_{10} , defined by them, nor the temperature, T_x , which they define using only the maximally compact ensemble², is precisely related to true thermodynamic stability. Furthermore, the correlation they observed between their energy gap and folding kinetics is only a weak trend, and the energy-gap condition they use is not sufficient to discriminate between folding and non-folding sequences (see Fig. 7 of ref. 2). In general, folding rate depends on the entire energy landscape³⁻⁶, not just the energy gap in a highly restricted ensemble.

Ultimately, the Levinthal problem is not that a protein has too many degrees of freedom. It is the shape, not the size alone, of the conformational energy landscape that matters^{4-6,9}. Many large land-

scapes have shapes that can be quickly traversed to reach the bottom⁶⁻⁹. Šali *et al.* show that Metropolis Monte Carlo sampling can find the lowest energy of a particular parameterized potential function, but this was already clear from many earlier efforts^{10,11}. The issue, therefore, is whether their potential function is better than earlier models. Baldwin in News and Views¹² has said that Šali *et al.* were using a potential function of the Miyazawa-Jernigan type, picked from the pairwise interactions in the protein database. But, as Šali *et al.* have noted¹, the terms are picked from a random gaussian distribution, not from the databank. Their potential function is not particularly physical, as correlations among contact energies of different pairs of amino-acid residues are neglected. It is unclear whether the potential is any more or less protein-like than any of the potentials used in previous works.

Baldwin¹² describes the work of Šali *et al.* as an important “new view” of protein folding. Naturally, lattice models are useful for addressing general physical principles of protein folding, even though they involve considerable simplification. However, it is clear from many earlier efforts, including some that used comparable lattice simulations, that many of the ideas Baldwin cites as “new” are already in the literature (refs 4, 10, 13–18, and refs therein, and reviewed more recently in refs 3, 5, 6).

Hue Sun Chan

Department of Pharmaceutical Chemistry,
University of California at San Francisco,
California 94143-1204, USA

This note was written with the assistance and concurrence of: Joseph D. Bryngelson, NIH, Bethesda, Maryland 20892, USA; Carlos J. Camacho, Facultad de Física, PUC, Casilla 306, Santiago 22, Chile; Ken A. Dill, Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143-1204, USA; José N. Onuchic & Nicholas D. Socci, Department of Physics, University of California at San Diego, La Jolla, California 92093-0319, USA; D. Thirumalai, Institute for Physical Science and Technology, University of Maryland, College Park, Maryland 20742, USA; and Peter G. Wolynes, School of Chemical Sciences, University of Illinois, Urbana, Illinois 61801, USA.

KARPLUS *ET AL.* REPLY — Chan *et al.* raise several questions, all of which have simple answers. The object of our study^{1,2} was to examine a large number of sequences and to separate those that fold from those that do not. Consequently, a temperature slightly above T_m the midpoint of the folding transition, was used to speed up the reaction. If folding to the native state were always possible under the simulation conditions, as Chan *et al.* imply there would not have been any non-folding sequences and our computer experiment would have failed. But this is not the case. Further, the same folding kinetics is observed throughout the temperature range where the true native state stability varies from 1 to 40%¹⁹.

A pronounced energy gap between the native and first excited state (equations

(3) and (10) in ref. 1) for the fully compact ensemble, is a necessary and sufficient condition for rapid folding in the model study. It is necessary because no sequence without such a minimum folds to the native state, and is sufficient because all sequences with such a minimum do fold (Fig. 7 of ref. 2). As to the 11 out of 200 sequences that have their minimum outside the fully compact set, none satisfied the energy condition nor did they fold repeatedly either to the lowest fully compact state or to the lowest energy state found by a Monte Carlo simulation. Thus, these sequences confirm and generalize the folding criterion². Further, the use of the energy condition for quantitatively determining the folding rate has been demonstrated²⁰. There is a strong correlation between the results from the fully compact states and the complete set of states (ref. 2 and Fig. 17 therein).

The nature of the configuration space, as well as the number of conformers, is important for the Levinthal paradox^{1,2}. Surfaces can be constructed for which resolution of the paradox is trivial, but this is not true for the 27-mer since only a fraction of sequences fold rapidly. The large size of the configuration space is necessary for the existence of a paradox. The 27-mer model has 10^{16} configurations and requires fewer than 5×10^7 Monte Carlo steps to find the native state. Short oligomers that have been extensively studied on a two-dimensional square lattice^{16,21} may be too small; for example, more Monte Carlo steps (10^5 or more) than there are configurations (4×10^4) were required for folding a 13-mer¹⁶.

The aim of the lattice simulations was to use random interactions so as to determine what differentiates folding from non-folding sequences. The exact choice of parameters was not important, as long as a reasonable set was used. The 27-mer parameters² correspond to the Miyazawa and Jernigan set²² in terms of the magnitude of the interaction energies and their standard deviation.

As to Baldwin's statement in News and Views that we presented a “new view” of protein folding, we agree that some of the concepts in refs 1 and 2 were presaged in earlier work of Go and Abe²³ and of others cited in refs 1 and 2. The 27-mer model studies^{1,2,18} provided the first demonstration that the energy-gap condition and a detailed mechanism for resolving the Levinthal paradox could be found *a posteriori* in computer experiments without having to be introduced explicitly *a priori* to achieve folding²⁴.

M. Karplus

A. Šali

E. Shakhnovich

Department of Chemistry,
Harvard University,
Cambridge, Massachusetts 02138, USA

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