## Reply to Comment on "Criterion that Determines the Foldability of Proteins"

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## Abstract

We point out that the correlation between folding times and  $\sigma = (T_{\theta} - T_f)/T_{\theta}$  in protein-like heteropolymer models where  $T_{\theta}$  and  $T_f$  are the collapse and folding transition temperatures was already established in 1993 *before* the other presumed equivalent criterion (folding times correlating with  $T_f$ alone) was suggested. We argue that the folding times for these models show no useful correlation with the energy gap even if restricted to the ensemble of compact structures as suggested by Karplus and Shakhnovich.

In a recent article [1] we (KT) showed that for certain lattice models of proteins the folding times,  $\tau_f$ , correlate extremely well with  $\sigma = (T_\theta - T_f)/T_\theta$  where  $T_\theta$  and  $T_f$  are well defined thermodynamic collapse and folding transition temperatures, respectively. We also demonstrated that there is "no useful correlation between folding times and the energy gap between the native conformation and the first excited state" [1]. In response to these results Karplus and Shakhnovich (KS) [2] try to argue (i) that the folding criterion used by KT is "essentially the same as one introduced earlier [3,4]" and (ii) that the energy gap used by KT is not "appropriate". We take up these two issues separately. In addition, we also show that folding times do not correlate with the energy gap  $\Delta_{CS}$  restricted to the ensemble of compact structures as KS [2] desire. First we settle the historical claims to priority. The criterion used by KT was already established in 1993 in [5] which was published before [4] was even received for review. There is no plot of folding time versus  $T_f$  in [3], an article that was submitted after [5] was accepted for publication. It was shown in [5] (cited in [4]) that folding times correlate with  $\sigma$ . We showed using numerical results and theoretical arguments that "there appears to be useful correlation between folding time  $\tau_r$  and  $\sigma = 1 - T_f/T_{\theta}$ : the smaller the value of  $\sigma$  the smaller the value of  $\tau_r$ " [5].

Having addressed the historical claim to priority we now examine the statement that our folding criterion is "essentially the same as one introduced earlier" [4]. This claim is based on the observation that there is a correlation between folding time and  $T_f$  seen in "right most part of Fig. (8a) of [4]". Fig. (8a) of [4] shows the folding time for 10 sequences of which 5 lie in the "right most part". The simulation temperature for the data in Fig. (8a) is  $T_s = 1.0$  and the  $T_f$  for all the sequences lie in the range  $0.63 \leq T_f \leq 1.06$  (see Table I in [4]). Of the 5 sequences that lie in the "right most part" of Fig. (8a) only one has  $T_f > T_s$ . The apparent correlation (for the five sequences for which the folding times merely change by a factor of 3) claimed by KS occurs when the native state is not stable. In Fig. (8b) in [4] a plot of folding time as a function of  $T_f$  at  $T_s = 0.7$  (which is below  $T_f$  for all sequences except two) is shown. In this figure, with  $T_f > T_s$ , one observes no correlation whatsoever between  $\tau_f$  and  $T_f$ . In contrast, our results [1] show that  $\tau_f$  (which spans six orders of magnitude) correlates well with  $\sigma$ . It is the interplay between the two intrinsic sequence dependent temperatures  $T_{\theta}$  and  $T_f$  that seems to correlate with  $\tau_f$  under conditions when the native state has the highest occupation probability.

It was forcefully stated in [6,7] that the "necessary and sufficient" condition for folding in these models is that there should be "a pronounced energy gap between the native and first excited state for the fully compact ensemble" [7]. The reason we did not display  $\tau_f$ as a function of  $\Delta_{CS}$  in [1] is that roughly half of the sequences had non-compact native conformations. It is, therefore, not clear why one should be restricted only to the ensemble of compact structures. However, the folding times for sequences in [1] when plotted as a function of  $\Delta_{CS}$ , as KS desire, also does not show any correlation (see Fig. (1a)). There are a large number of sequences, all with roughly the same folding times, but with very different values of  $\Delta_{CS}$ . The lack of correlation between the energy gap and foldability has been established for other models as well [9–11].

There are a few additional comments that are worth making. (1) KS claim that  $\tau_f$  should correlate well with both  $T_f$  and  $\Delta_{CS}$ . This implies that  $T_f$  should correlate with  $\Delta_{CS}$ . In Fig. (1b) we plot  $T_f$  as a function of  $\Delta_{CS}$ . The lack of correlation is evident. (2) Unlike  $T_{\theta}$ and  $T_f$ ,  $\Delta_{CS}$  is not experimentally measurable. Moreover, with the exception of two studies [10,12], the energy gap is not easily computable for simple off-lattice models. Thus the practical utility of  $\Delta_{CS}$  is not clear. (3) We should emphasize that all the criteria proposed for folding times should be viewed as statistical. This means that, under conditions when the native state is significantly populated,  $\tau_f$  may correlate with certain properties intrinsic to the sequence provided a number of sequences is studied. Our results suggest that  $\sigma$  could be one of such properties.

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## FIGURES

Fig. 1. (a) Folding time  $\tau_f$  versus  $\Delta_{CS}$ . (b)  $T_f$  as a function of  $\Delta_{CS}$ . Solid circles correspond to 15-mer and open circles are for 27-mer. Right and upper axes are for 27-mer.

