LETTER

REPLY TO EATON AND WOLYNES: How do proteins fold?

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Eaton and Wolynes (1) take issue with our recent paper on protein folding (2) in which we compare the defined-pathway model (3, 4) and the many-pathway funneled-energy landscape model (5, 6) with informative experimental results.

Eaton and Wolynes (1) do not dispute our main points. Over a dozen proteins have now been shown to be constructed of separately cooperative foldon units. They fold through foldon-dependent intermediates in well-defined pathways. Multiple alternative pathways, if they existed, would have been seen in these experiments; they have not. The multiple trajectories visualized in the funneled-landscape model have finally been experimentally observed. In single-molecule experiments (7), a tethered prion dimer held in dual optical traps under constant pulling force was seen to flip back and forth between the unfolded state and the first of three defined folding-pathway intermediates. The results were claimed by Wolynes to confirm the many-pathway model (8). Our paper shows that the many kinetic trajectories are exponentially distributed as for any simple molecular kinetic process, and their multiple parallel nature has no special influence on speeding macroscopic folding (2). More generally, we show that amino acid-level conformational searching does not have the energetic bias required by Zwanzig et al. (9) or the structural discrimination needed to support realistic protein folding. The collective energetics and native-like character provided by the foldon hypothesis automatically solves these difficulties.

Mainly, in their letter Eaton and Wolynes (1) advocate the value of theoretical approaches. We agree with this view. However, Eaton and Wolynes unfortunately continue to conflate the funnel model and its multipathway focus with the entire theoretical enterprise. We do not agree that much current theory convincingly supports multiple folding pathways. Physics-based atom-level simulations find that proteins fold in a defined reproducible stepwise pathway (10), as do other promising approaches. The much more chaotic microscopic dynamics, also observed, are a universal correlate of any diffusional process and should not be confused with multiple folding pathways. The experimental references noted by Eaton and Wolynes (1) that suggest off-pathway steps or an alternative folding mode at some nonnative condition does not provide strong support for the proposition of countless pathways.

The multipathway idea stems from the early presumption that structure formation must occur through microscopic amino acid-level searching. The funneled-landscape picture itself does not dictate whether pathways are well-defined or multiple. Unlike an authentic energy landscape, the usual funnel-shaped landscape simply expresses some obvious thermodynamic constraints equally applicable to proteins, RNA, and any other polymer. Folding must proceed energetically downhill and lose conformational entropy as it goes.

The field has profited from funneled-energy landscape theory and we congratulate its creators, but we suggest that it is time to move on. Recent progress suggests a different vision.

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