LETTER

REPLY TO ALBERTI:
Are in vitro folding experiments relevant in vivo?

Pavel I. Zhuravlev[a,1], Michael Hinczewski[b], Shaon Chakrabarti[a], Susan Marqusee[c], and D. Thirumalai[a,1]

In his letter, Alberti (1) does not challenge any of the central results in our paper (2), including the main proof that upward curvature in the logarithm of the unfolding rate of a protein as a function of an applied mechanical force implies that underlying energy landscape is multidimensional. However, he wonders if the switch in pathway in Src tyrosine kinase SH3 (Src homology 3) domain discovered in single-molecule pulling experiments (3, 4) applies to "living cells/physiological settings," an issue that is not germane to our work (2), and, by inference, to innumerable in vitro ensemble (5–7) and single-molecule studies on proteins. Although Alberti's view point has merits, it can be asserted that such biophysics studies and countless others have literally revolutionized modern biology.

A few other points are worth making. (*i*) Previously, we showed that there are two unfolding pathways even if F is applied between the N and C termini (8). The same inferences were drawn by noting upward curvature in the denaturant-dependent unfolding rates of I27 (6) and monellin (7). (*ii*) The major reason why Alberti questions the relevance of several experiments, which have used multiple pulling directions to map the folding landscape of proteins, is that in the proteins he lists (1), the N and C termini are solvent-exposed. Although this fact generally appears to be correct, there are a number of proteins in which this fact is not the case (9). (*iii*) The proposed link (1) between chaperones and pulling experiments is even more tenuous. The mechanism of how eukaryotic Hsp 70 interacts with substrate proteins (SPs) has not been quantitatively elucidated. In bacterial chaperonin (GroEL/GroES), we showed that SPs could experience an unfolding force of $\approx$ 10 pN (10) [confirmed recently (11)] due to ATP-driven GroEL domain movements. It is unlikely that this force is directed along the N and C termini of the misfolded SP. (*iv*) The transit through the tunnel of the ribosome is facilitated by the nascent protein experiencing a mechanical force. However, because the translational rate is slow, force direction on the protein could vary, especially as it reaches the vestibule of the ribosome. Subsequent folding is a complicated process involving trigger factor and folding in confined spaces, with force being irrelevant.

It is a matter of opinion whether precise in vitro studies are valuable in understanding physiological processes. Only by using theory, experiments, and simulations in well-defined systems can quantitative understanding of the functions of proteins under cellular conditions be achieved.

1 Alberti S (2016) Native, sequential protein folding via anchored N and C protein termini. *Proc Natl Acad Sci USA* 113:E3189–E3191.
2 Zhuravlev PI, Hinczewski M, Chakrabarti S, Marqusee S, Thirumalai D (2016) Force-dependent switch in protein unfolding pathways and transition-state movements. *Proc Natl Acad Sci USA* 113(6):E715–E724.
3 Jagannathan B, Elms PJ, Bustamante C, Marqusee S (2012) Direct observation of a force-induced switch in the anisotropic mechanical unfolding pathway of a protein. *Proc Natl Acad Sci USA* 109(44):17820–17825.
4 Guinn EJ, Jagannathan B, Marqusee S (2015) Single-molecule chemo-mechanical unfolding reveals multiple transition state barriers in a small single-domain protein. *Nat Commun* 6:6861.
5 Fersht A (1998) *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding* (W. H. Freeman Company, New York).
6 Wright CF, Lindorff-Larsen K, Randles LG, Clarke J (2003) Parallel protein-unfolding pathways revealed and mapped. *Nat Struct Biol* 10(8):658–662.
7 Aghera N, Udgaonkar JB (2012) Kinetic studies of the folding of heterodimeric monellin: Evidence for switching between alternative parallel pathways. *J Mol Biol* 420(3):235–250.
8 Zhuravlev PI, Reddy G, Straub JE, Thirumalai D (2014) Propensity to form amyloid fibrils is encoded as excitations in the free energy landscape of monomeric proteins. *J Mol Biol* 426(14):2653–2666.
9 Jacob E, Unger R (2007) A tale of two tails: Why are terminal residues of proteins exposed? *Bioinformatics* 23(2):e225–e230.
10 Thirumalai D, Lorimer GH (2001) Chaperonin-mediated protein folding. *Annu Rev Biophys Biomol Struct* 30:245–269.
11 Corsepius NC, Lorimer GH (2013) Measuring how much work the chaperone GroEL can do. *Proc Natl Acad Sci USA* 110(27): E2451–E2459.

[a]Biophysics Program, Institute for Physical Science and Technology, Department of Chemistry & Biochemistry, University of Maryland, College Park, MD 20742; [b]Department of Physics, Case Western Reserve University, Cleveland, OH 44106; and [c]California Institute for Quantitative Biosciences, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

Author contributions: P.I.Z., M.H., S.C., S.M., and D.T. designed research; P.I.Z., M.H., S.C., S.M., and D.T. performed research; D.T. contributed new reagents/analytic tools; D.T. analyzed data; and D.T. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence should be addressed. Email: dave.thirumalai@gmail.com.