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Probing the Mechanical Unfolding of Single Protein Molecules at High Resolution

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ABSTRACT

A major drive in protein folding has been to resolve the myriads of microscopic pathways and complex mechanisms that purportedly underlie the simple folding behavior observed in conventional experiments. This is key for cross-validating predictions from theory and modern computer simulations. Over the last 15 years two synergistic routes have been explored: one focuses on improving methods to increase the time, structural or single-molecule resolution, whereas the other targets proteins that fold ultrafast and thus with the limited cooperativity that makes the underlying complexity more evident. One technique that is particularly attractive is force spectroscopy by which an individual protein is mechanically controlled to simultaneously induce and observe its unfolding and refolding. The problem has been how to reach the required fast time resolution and the fine control of force to fully resolve (un)folding. Here I will discuss our recent results using enhanced atomic force microscopy (AFM) to mechanically unfold two different proteins at moderate loads: cold shock protein B (Csp), a showcase of cooperative two-state folding; and gpW, a protein that in isolation folds in a few microseconds over a marginal folding barrier (~1 kBT).

On the other hand, experiments on the ultrafast folder gpW allow us to resolve, for the very first time on an AFM, a single protein molecule alternating stochastically between its folded and extended states. The big surprise is that the folding-unfolding of gpW occurs at 10,000-fold slower times than in absence of force. Using theory and molecular simulations we rationalize these striking results as arising from a modest, force-induced free energy barrier, and a drastic slowdown of the overall dynamics caused by linkage to the AFS cantilever that enables to capture the fast folding and unfolding events of this protein in slow motion. Our experiments on the two-state folder Csp, show unexpected unfolding heterogeneity with trajectories ranging from single sweeps to different combinations of multiple long-lived mechanical intermediates that also vary in order of appearance. Steered molecular dynamics simulations closely reproduce the experimental observations, thus matching unfolding patterns with structural events. Our results on Csp provide, for the first time, a direct glimpse at the nanoscale complexity underlying two-state folding.

BIO:

Victor Muñoz is currently a Professor of Bioengineering at the University of California Merced. He carried out his PhD at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, and postdoctoral work at the National Institutes of Health in Bethesda, Maryland, as a Human Frontiers Science Program Postdoctoral Fellow. From 2000 to 2007 he was Assistant and Associate Professor at University of Maryland, were he received the Camille and Henry Dreyfus New Faculty Award, the Packard Fellowship, and the Searle Scholarship. From 2007 to 2014 he was a Research Professor at the National Research Council (CSIC) in Madrid, Spain, where he was elected member of the European Molecular Biology Organization (EMBO) and received an Advanced Grant from the European Research Council (ERC). He joined the faculty at UC Merced in 2014 where he is currently Co-Director of the Cellular and biomolecular Machines NSF-CRESt center and recipient of an award from the Keck foundation. His research interests involve the investigation of protein folding, protein engineering and de novo design, both from the fundamental and applied standpoints. One of the most distinctive characteristics of his research career is the deployment of a profoundly multidisciplinary approach that combines experiment and theory using Molecular Biology-Biochemistry, Physics, Chemistry and Computer Science with a focus on solving problems of biomedical and biotechnological relevance and developing novel bioengineering applications.