The puzzling folding behavior of protein gankyrin.

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Gankyrin is a tandem-repeat protein, formed by seven ankyrin repeats, presenting intriguing equilibrium and kinetics behaviors. The modularity and the size of the protein prompt for the possible existence of equilibrium and kinetics intermediates, and alternative relaxation pathways, on top of a highly complex energy landscape, most of which invisible to direct experimental observation. We address the modeling of the folding behavior of gankyrin using a simple native-centric model, capable of reproducing the main features of the experimental results and to provide some insight into those aspects of the folding/unfolding process that are not directly accessible by experimental techniques.

Indeed, experiments reveal that the 7-ankyrin repeat protein gankyrin folds and unfolds via two alternative pathways [1] (Fig. 1), where the structure unravels progressively from one or other end of the molecule. Single-site mutants shift the relative flux through the pathways. It is possible to see that pathway B has a broad rate-limiting energy barrier whereas pathway A has a rather narrow rate-limiting energy barrier.

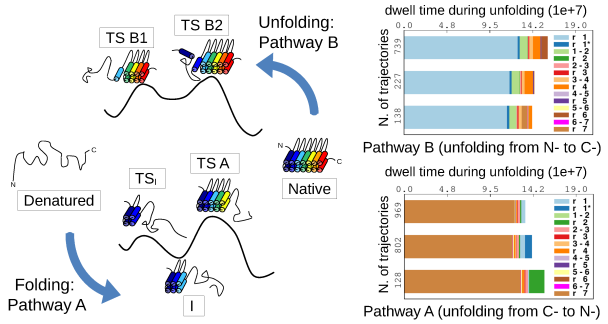

Figure 1: Left: schematic representation of the folding and unfolding behavior or protein gankyrin. Right: dwell time in different structures along unfolding pathways A and B

A striking finding is that gankyrin folds along a different route from that along which it unfolds; i.e. what folds first does not unfold last. In the folding direction the N-path is favored: structure starts accumulating at the N-term and builds up towards the C-term; notably, the N-terminal region is also the most stable at equilibrium. However, when it comes to the unfolding reaction at high denaturant, it is the N-terminal region that dissociates the more easily from the native repeat stack. We find that a model based purely on the native contacts is able to reproduce the key features of the energy landscape obtained by experiment, predicting the dominance of N-polarized structure in equilibrium intermediates, the existence of two unfolding pathways involving sequential unraveling from either end of the protein chain, and the flux shift between pathways in the unfolding reaction. Finally, the simulations of the model shed light on key features of the energy landscape of gankyrin that are not accessible to experiment: they provide a framework within which to reconcile the seemingly contradictory behavior of the protein under equilibrium/refolding conditions (i.e. high stability of the N-terminal part of the polypeptide chain) when compared with kinetic unfolding conditions (i.e. dominance of the unfolding pathway involving unravelling from the N-terminus).

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[1] R. D. Hutton, J. Wilkinson, M. Faccin, E. M. Sivertsson, A. Pelizzola, A. R. Lowe, P. Bruscolini and L. S. Itzhaki. *"Mapping the Topography of a Protein Energy Landscape"*, J. Am. Chem. Soc. **137**, 14610 – 14625 (2015).