456f Unfolding a Linker between Helical Repeats

Vanessa Ortiz, Steven O. Nielsen, Michael L. Klein, and Dennis E. Discher

In many multi-repeat proteins, linkers between repeats have little secondary structure and place few constraints on folding or unfolding. However, the large family of spectrin-like proteins – including α-actinin, spectrin, and dystrophin – share 3-helix bundle, spectrin repeats that appear in crystal structures to be linked by long helices. All of these proteins are regularly subjected to mechanical stress. Recent single molecule AFM experiments demonstrate not only forced unfolding but also simultaneous unfolding of tandem repeats at finite frequency, which suggests the contiguous helix between spectrin repeats can propagate a cooperative helix-to-coil transition. Here we address what happens atomistically to the linker under stress by steered molecular dynamics (SMD) simulations of tandem spectrin repeats in explicit water. A new way of assessing conformational changes in proteins is introduced, which involves calculating the deformations or ‘strains' in the molecule relative to its native or equilibrium state. The results for α-actinin repeats reveal rate-dependent pathways, with one pathway showing that the linker between repeats unfolds, which may explain the single-repeat unfolding pathway observed in AFM experiments. A second pathway preserves the structural integrity of the linker, which explains the tandem-repeat unfolding event. Unfolding of the linker begins with a splay distortion of proximal loops away from hydrophobic contacts with the linker. This is followed by linker destabilization and unwinding with increased hydration of the backbone. The end result is an unfolded helix that mechanically decouples tandem repeats. Molecularly detailed insights obtained here aid in understanding the mechanical coupling of domain stability in spectrin family proteins.