

MECHANICAL ARCHITECTURE OF THE TANDEM IMMUNOGLOBULIN REGIONS OF TITIN STUDIED BY SINGLE MOLECULE AFM

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The elastic I band region of human cardiac titin contains two distinct Ig regions containing 15 (proximal) and 22 (distal) Ig domains placed in tandem and separated by the N2B and PEVK unique sequences. Here we combine protein engineering with single molecule AFM to examine the mechanical properties of the distal and the proximal Ig regions. The proximal domains all have a similar mechanical stability requiring ~ 170 pN of force to unfold when pulled at a rate of 600 nm/s. By contrast the distal domains are on average more stable than the proximal ones and show a strong hierarchical unfolding starting at ~150 pN and up to 300 pN. While the proximal Ig domains undergo simple two-state unfolding, the distal Ig domains unfold through an intermediate state. This intermediate has the effect of protecting the distal Ig modules against mechanical unfolding. In the steady state, a force of only 10 pN unfolds most of the proximal domains and none in the distal region. The difference in the mechanical properties of these two regions may reflect the mechanical topology of these two classes of Ig modules and explain the different extensibility of the two Ig regions observed in single myofibril experiments.