STEPWISE UNFOLDING OF TITIN UNDER FORCE-CLAMP AFM

Andres F Oberhauser[1], Paul K Hansma[2], Mariano Carrion-Vazquez[1], Julio M Fernandez[1], : [1]Mayo Foundation, Rochester, MN 55905, [2]University of California Santa Barbara, CA

The mechanical extension of the muscle protein titin is thought to be due to probabilistic unfolding events that are dependent on the stretching force. Here we demonstrate the implementation of a single molecule force-clamp adapted for use with an AFM that we use to directly test this hypothesis. Force-clamp recordings give exceptionally clean records of the mechanical unfolding of titin. We show that under force-clamp conditions, an engineered titin protein elongates in steps due to the unfolding of its modules (see figure) and that the waiting times to unfold are exponentially distributed, as predicted by a simple first order kinetic model. We also show that the unfolding probability depends sigmoidally on the applied force. Single molecule force clamp measurements, as demonstrated here permit, for the first time, direct measurements of the force-driven unfolding kinetics of a single protein. This new form of single protein force spectroscopy promises to be a direct way to probe elastic proteins such as those found in muscle, the extracellular matrix and in cell adhesion.