MECHANICAL UNFOLDING OF NATIVE FIBRONECTIN USING THE ATOMIC FORCE MICROSCOPY

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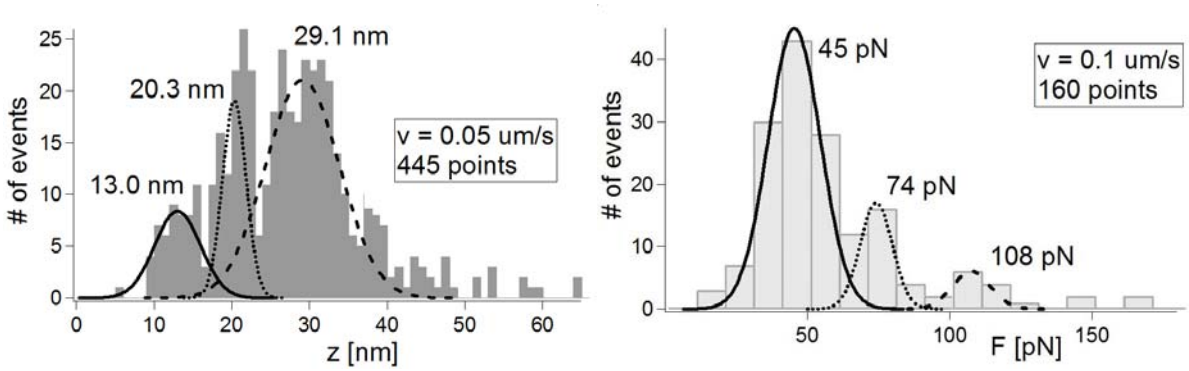
The structure of a particular protein is encoded in its linear sequence of amino acid residues. All proteins are synthesized on the ribosome where each protein molecule must fold into the specific conformational state in order to be able to carry out its biological function. The native protein conformation is not always stable. Very often even small changes in the protein's environment may lead to structural changes that can affect protein biological function. Therefore, the investigation of the transition between the folded and unfolded states is one of the challenging problems in structural biology [1].

The atomic force microscopy (AFM) was applied to unfold the native fibronectin coming from bovine plasma. Fibronectin is known to mediate a number of biological interactions between cells and extracellular matrix, including cell adhesion and cell motility. The fibronectin consists of three types of domains: FnI, FnII and FnIII. Each domain (in a full unfolded state) has a characteristic length of about 45, 60 and 90 amino acids, respectively. In spite of wide AFM application to protein unfolding, only domain type III was the most studied and the best characterized [2].



Length (left) and force (right) histograms obtained from the unfolding of the native fibronectin. Gauss functions were fitted in order to determine the length and force characteristic for the unfolded fibronectin domains (FnI, FnII, FnIII).

The unfolding of the native fibronectin showed the contribution of three domains indicating that in spite of disulfide bonds stabilizing FnI and FnII, the partial unfolding of these domains was observed, which was verified by the use of the reducing agent DTT. The influence of the pulling speed (ranging from 0.05 to 0.5 μm/s), buffer pH value and selection criteria for curves were investigated.

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[2] A. F. Oberhauser, C. Badilla-Fernandez, M. Carrion-Vazquez, J. M Fernandez, J. Mol. Biol. **319** (2002) 433.