Unfolding pathway of the cancer-associated NQO1 enzyme studied at the single molecule level.

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NQO1 is a FAD-dependent NAD(P)H quinone oxidoreductase that activates cancer pro-drugs and stabilizes oncosuppressors such as p53 and p73. Disease associated mutations usually result in intracellular enzyme inactivation, dysregulation and instability. It has been previously shown that the mutant P187S strongly destabilizes the NQO1 dimer in vitro, resulting in a loss-of-function and cancer-associated polymorphism. However, a combination of FAD and the inhibitor Dicoumarol seems to revert these alterations. It has been suggested that FAD and Dicoumarol stabilize NQO1 against degradation. Here we apply nanopore force spectroscopy to analyze the stability of the protein against vectorial unfolding. We found that addition of FAD and Dicoumarol changes the unfolding pathway resulting in slower unfolding, which may explain the lower degradation rate observed *in vivo*. Our results suggest that protein unfolding through nanopores capture the basics of protein unfolding by the proteasome in the absence of ubiquitination.