

# Pulling Direction as a Reaction Coordinate for the Mechanical Unfolding of Single Molecules

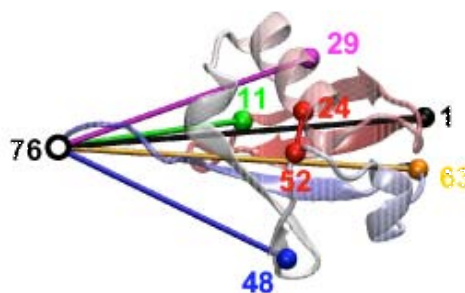
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Recent advances in experimental techniques enable researchers to exert forces on individual molecules and observe their response in real time. In these experiments, single molecules – such as proteins and nucleic acids – are pulled apart by atomic force microscope, laser optical tweezers, or using related techniques.

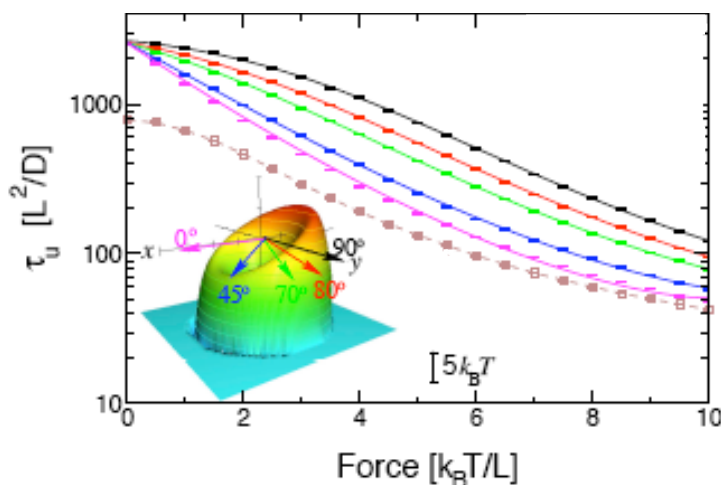
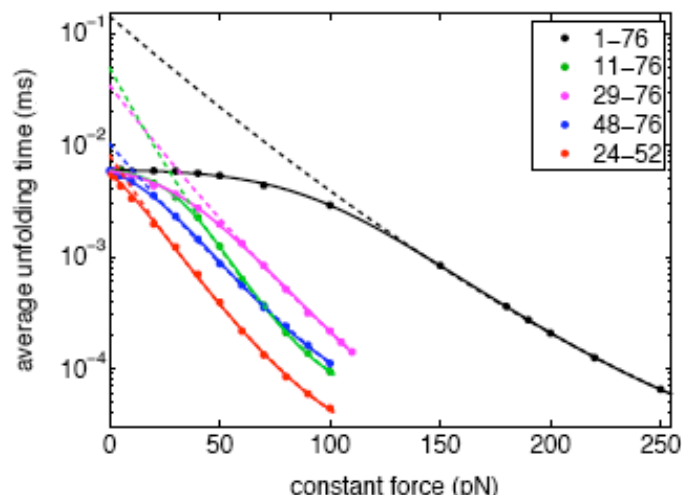
Folded and unfolded states of proteins or large RNAs are usually separated by high free energy barriers. Consequently, extracting intrinsic unfolding kinetic information, such as unfolding rates at zero force, requires an extrapolation from the data collected at a large pulling force, by fitting a theoretical model.

It is frequently said that mechanical unfolding experiments have a “well-defined” reaction coordinate, since the pulling coordinate is clearly that along which the force acts. But is it also a good reaction coordinate? Is it relevant to the intrinsic folding pathway and therefore are the fitted parameters relevant descriptors of the unfolding reaction? Our results demonstrate that, at least for the protein ubiquitin model used in this study, most pulling coordinates are not good reaction coordinates for unfolding at zero force because they produce extrapolated rates which differ from the “true” rates.

**Fig.1** The five naturally occurring pulling coordinates for ubiquitin. The pulling coordinate is defined here as the molecular extension on which the pulling force acts, e.g., the distance between the ends of a protein if it is pulled from the termini



Unfolding rates calculated from simulations of ubiquitin over a broad range of stretching forces, for different pulling directions, reveal a remarkable turnover from a force-independent process at low force to a force-dependent process at high force. The turnover can be explained by a switch from the intrinsic barrier probed by pulling at very low forces, to a different barrier at high force.



**Fig.2** (Left) Dependence of unfolding time on pulling force for different pulling directions. For all of the pulling coordinates the curves show a “turnover” to a force-independent process, meeting at zero force. A novel pulling coordinate 24-52, designed to be the most relevant to the intrinsic folding pathway, results in the smallest turnover. (Right) Turnover can occur for dynamics on minimalist 2D surfaces

We use the turnover to suggest an experimental criterion to determine the pulling coordinate which best describes the intrinsic unfolding. Such a coordinate represents the “Achilles’ heel” of the protein, that is to say, pulling on it would be the most effective at accelerating the unfolding of the protein and yield parameters most relevant to the intrinsic folding barrier.