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Force feedback effects on single molecule hopping and pulling experiments

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Single-molecule experiments with optical tweezers have become an important tool to study the properties and mechanisms of biological systems, such as cells and nucleic acids. In particular, force unzipping experiments have been used to extract the thermodynamics and kinetics of folding and unfolding reactions. In hopping experiments, a molecule executes transitions between the unfolded and folded states at a preset value of the force [constant force mode (CFM) under force feedback] or trap position [passive mode (PM) without feedback] and the force-dependent kinetic rates extracted from the lifetime of each state (CFM) and the rupture force distributions (PM) using the Bell-Evans model. However, hopping experiments in the CFM are known to overestimate molecular distances and folding free energies for fast transitions compared to the response time of the feedback. In contrast, kinetic rate measurements from pulling experiments have been mostly done in the PM while the CFM is seldom implemented in pulling protocols. Here, we carry out hopping and pulling experiments in a short DNA hairpin in the PM and CFM at three different temperatures (6 °C, 25 °C, and 45 °C) exhibiting largely varying kinetic rates. As expected, we find that equilibrium hopping experiments in the CFM and PM perform well at 6 °C (where kinetics are slow), whereas the CFM overestimates molecular parameters at 45 °C (where kinetics are fast). In contrast, nonequilibrium pulling experiments perform well in both modes at all temperatures. This demonstrates that the same kind of feedback algorithm in the CFM leads to more reliable determination of the folding reaction parameters in irreversible pulling experiments. *Published by AIP Publishing.* https://doi.org/10.1063/1.5010303

I. INTRODUCTION

The invention of single-molecule manipulation techniques over the past decades has provided new insights into the details of complex molecular reactions in cells[1–5] that complement traditional bulk methods. Techniques such as optical tweezers[6,7] allow scientists to mechanically unzip and stretch single biomolecules like DNA,[8–10] RNA,[11,12] and proteins[13–15] in a controlled manner.

In DNA unzipping experiments, a tensile force is applied to the 3′ and 5′ of a DNA hairpin until the base pairs (bp's) that stabilize the double helix are disrupted and the native hairpin unfolds and is converted into single-stranded DNA (ssDNA).[10,16,17] The unzipping experiment is the equivalent of a temperature-induced melting process, the main difference being the final state of the DNA hairpin: a stretched polymer in the former versus a random coil in the latter. The reverse of the unzipping process is molecular folding or zipping: upon releasing the force, the stretched ssDNA folds back into the native hairpin in a process determined by the nucleation of the loop and the formation of the stem. The thermodynamics and kinetics of the unzipping-zipping reaction give valuable information about the free energy of folding of the DNA hairpin and the underlying molecular free energy landscape otherwise difficult to obtain in bulk assays. The unfolding and folding reaction can be studied in two types of force spectroscopy protocols using optical tweezers: hopping and pulling. Moreover, experiments can be carried out in two control modes: constant-force mode (CFM) and passive mode (PM) depending on whether the force (CFM) or the trap position (PM) is the control parameter (i.e., the externally controlled variable that can be held fixed and is not subject to thermal fluctuations).

In hopping experiments, the control parameter (trap position or force) is kept constant while the molecule executes transitions between the folded and unfolded states.[18–21] In contrast, in pulling experiments, the control parameter is repeatedly and continuously changed between two limit values while the force-distance curve is recorded along the stretching-releasing cycles.[22,23] Single-molecule experiments monitor transitions in real time, allowing us to measure transiently fast and rare events that would otherwise be masked in bulk assays where only averages over a large number of molecules are measured.

The force-dependence of the measured unfolding and folding kinetic rates is typically studied using the Bell-Evans (BE) model[24,25] where the unfolding-folding process is a thermally activated diffusive process that passes through a transition state (TS) characterized by a kinetic barrier. Kinetic rates exhibit an Arrhenius-like exponential dependence where the kinetic barrier in the exponent changes linearly with force. Fitting the BE model to the experimentally measured kinetic rates allows us to estimate the distance from each state (folded and unfolded) to the transition state.[20,26] The BE model is based on the force ensemble, i.e., force is the control parameter in the experiment. In an optical tweezer setup,