Experimental free energy measurements of kinetic molecular states using fluctuation theorems

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Recent advances in non-equilibrium statistical mechanics and single molecule technologies make it possible to extract free energy differences from irreversible work measurements[1–3]. To date, free energy recovery has been focused on native or equilibrium states, whereas free energy measurements of kinetic states (i.e. finite lifetime states that are generated dynamically and are metastable) have remained unexplored. Kinetic states can play an important role in various domains of physics, such as nanotechnology or condensed matter physics. In biophysics, there are many examples where they determine the fate of molecular reactions: protein and peptide-nucleic acid binding, specific cation binding, antigen-antibody interactions, transient states in enzymatic reactions or the formation of transient intermediates and non-native structures in molecular folders. Here we demonstrate that it is possible to obtain free energies of kinetic states by applying extended fluctuation relations[4,5]. This is shown by using optical tweezers to mechanically unfold and refold DNA structures exhibiting intermediate and misfolded kinetic states[6].

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