Affinity measurements of ligands binding nucleic acids using fluctuation theorems

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Fluctuation theorems allow to relate the work performed along non-equilibrium trajectories to thermodynamic free-energy differences. In the past years, fluctuation theorems have been used to obtain the free-energy of formation of DNA and RNA structures from force-spectroscopy measurements[1]. More recently, an extended version of the Crooks fluctuation relation has been used to recover free-energies of intermediate and misfolded structures[2,3]. However, so far this method has only been applied to unimolecular reactions. In this work, we have measured the binding affinity of a set of ligands to DNA and RNA structures using the Crooks fluctuation relation. We have measured the affinity of binding of single DNA intercalators, DNA restriction enzymes, and an RNA binding protein, finding good agreement with bulk measurements. We have measured the binding energy of low solubility compounds difficut to characterize with bulk techniques. The use of fluctuation theorems to obtain binding affinities in bimolecular reactions is also of interest to characterize protein-protein affinities.

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