Free energy measurement of ligands binding nucleic acids using fluctuation theorems

Joan Camunas-Soler,[1] Anna Alemany,[1] and Felix Ritort[1, 2, 3]

[1] *Departament de Física Fonamental, Universitat de Barcelona, Diagonal 647, 08028 Barcelona, Spain*
[2] *CIBER de Bioingeniería, Biomateriales y Nanomedicina, Instituto de Salud Carlos III, Madrid, Spain*
[3] *fritort@gmail.com*

Fluctuation theorems allow to relate the work performed along non-equilibrium processes 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 developed a novel methodology based on fluctuation relations to determine the free energy of binding of peptides and proteins to nucleic acids, so essential in many regulatory processes and drug targeting. We performed pulling experiments of DNA hairpins containing a binding site for a given DNA binding ligand (Figure 1) and measured the irreversible work done in the experiments. We then used a new fluctuation theorem to extract the affinity of binding of the ligands (i.e. single DNA peptides, DNA restriction enzymes, and an RNA binding protein), finding a new method to extract chemical potentials. Using this fluctuation relation we have also measured the binding energy of low solubility compounds difficult to characterize with bulk techniques and that find application as anticancer agents[4]. Finally, this methodology should also be useful to determine the binding affinities of protein-protein interactions, so essential in multiprotein assembly.

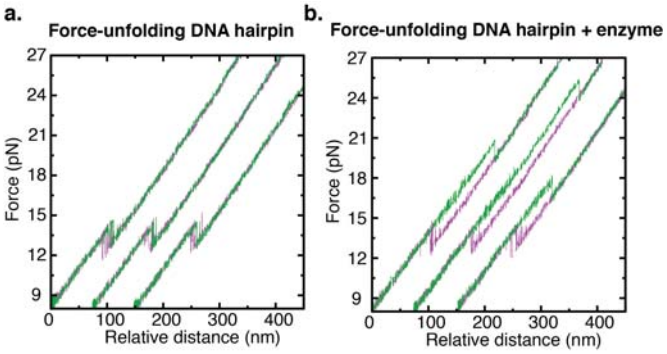

FIG. 1. Detection of binding events one-at-a-time (**a**) Pulling curves of a DNA hairpin in the absence of binding enzyme. Hairpin unfolding (green) and refolding (purple) is reversible and happens at a force range 13-14 pN. (**b**) Pulling curves of a DNA hairpin in the presence of an enzyme that binds at the hairpin stem. In the presence of protein the unfolding of the hairpin happens at a much higher force range of 21-26 pN due to the stabilizing effect of protein binding. When the force is released, the hairpin refolds at the typical hairpin coexistence force of 13 pN.

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