Physical picture for mechanical dissociation of biological complexes

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Since the irruption of single molecule techniques, the study of biological processes at the molecular level has been completely revolutionized. In particular, single molecule dynamic force spectroscopy (DFS) involves the application of controlled forces to biological macromolecules or complexes, inducing conformational changes such as unfolding of proteins [1] and nucleic acids or dissociation of ligand-receptor complexes [2]. In this context, processes such as molecular unbinding are usually characterized by a free energy profile along a reaction coordinate, namely the pulling direction. This profile shows a single -or multiple- free energy barrier $\Delta G^\dagger$ and an unbinding or dissociation free energy $\Delta G0$, which respectively control the kinetic and thermodynamic properties of the system. Ultimately, force spectroscopy experiments aim to access to this information by measuring rupture forces on such molecular transitions.

In the last few years, wide theoretical effort has been focused on this goal. For instance, when pulling a molecule at constant rate, force spectroscopy theory [3] relates the most probable rupture force with the free energy barrier height, the position of the transition state and the intrinsic rate coefficient. In addition, Jarzysnki equality [4] provides the free energy difference between two equilibrium states by measuring the work performed over a non-equilibrium transition between such states. A combined application of both theoretical frameworks would provide a global picture of the kinetic and equilibrium characteristics of the system. Nevertheless, they should be understood together within a suitable shape of the free energy profile, concerning the particular system of study.

In our work [5], we study the problem of mechanical dissociation of molecular complexes. Here, the force is typically exerted to the complex through a polymer linker attached to the pulling apparatus, which retracts at constant rate until the complex dissociates at a certain rupture force. From this magnitude, one should be able to derive the main properties of the underlying free energy profile. We use a simple analysis procedure, which allows for recovering the relevant free energy magnitudes $\Delta G^\dagger$ and ΔG^0 from rupture force measurements, by applying DFS theory and Jarzysnki equality. The method is applied to DFS-AFM experiments of the complexes formed by the flavoenzyme Ferredoxin-NADP+ reductase (FNR) and its two protein partners Ferredoxin (Fd) and Flavodoxin (Fld) from cyanobacterium Anabaena PCC 7119. This system is paradigmatic for the study of the association mechanism of redox complexes, and their interaction mechanism has been carefully characterized. We provide values for the barrier position and height, and for the dissociation free energies, which remarkably match the determined thermodynamic values. We also introduce a plausible model for the mechanical unbinding of biomolecular complexes that suits the experimental observations. To study the validity of the proposed model and the consistency of our method, we perform stochastic numerical simulations and analyse them with the same procedure used with the experimental data. Thus, on the one hand we validate the use of a simple and robust analysis protocol in order to jointly determine $\Delta G^\dagger$ and ΔG^0 from DFS experiments. On the other hand, we propose a free energy profile shape for mechanical dissociation of biomolecular complexes, focusing on the physical mechanism that drives this process.

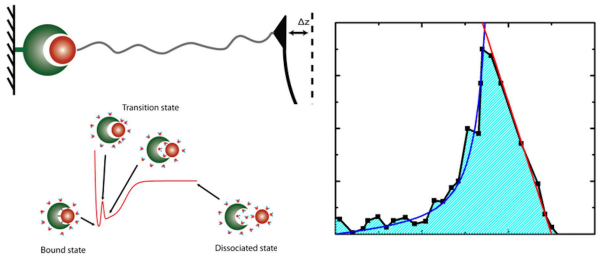

Figure 1: Proposed physical model for the studied unbinding of biological complexes.

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