Thermodynamic and kinetic analysis of a DNA hairpin using optical tweezers and a temperature controller

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In the last decades a large number of astonishing molecular nano-structures and nano-machines operating inside the cell have been discovered. Examples range from the DNA double helix, the most dense memory support in nature, to molecular motors, which are far more efficient than macroscopic machines. In the study of these objects, one of the most important new technologies are optical tweezers (OT).

OTs can trap and manipulate microscopic objects in a non-invasive way using light[1]. They consist of highly focused laser beams and can trap nano and microscopic dielectric objects, from neutral atoms to plastic microbeads. By trapping we mean the ability to exert forces on one object and thus to constrain its position in a certain region of space. For example we are able to exert force on a single cell, or to measure the force required to unfold the secondary structure of a double stranded deoxyribonucleic acid (dsDNA) molecule[2].

As temperature plays a key role in all biological processes, slight changes of temperature may lead to completely different behaviours of biological systems, or in our case the behaviour of a short DNA hairpin. In fact, living matter carry out its function in a small range of temperature. Therefore, it is interesting to study and understand what is the effect of temperature in biological systems. In this project we aim to understand and characterize the thermodynamic, kinetic and elastic response of a DNA hairpin at different temperature.

We have used the OT technique in order to measure and exert force in a DNA hairpin. Under the effect of a mechanical force at different temperatures we can perform two kinds of experiment: 1) Equilibrium (Hopping) experiment and 2) Non-equilibrium (Pulling) experiments.

A DNA hairpin is a single stranded deoxyribonucleic acid (ssDNA) that can fold into themselves to form a hairpin structure. This structure has two different part, the first one that is called stem is the region of the ssDNA that can form base-pairs (in our case 20 base-pairs), it means that when the DNA hairpin is folded the stem forms a dsDNA. The other part of the hairpin is called loop. The loop is the region of the ssDNA that can not form base-pairs, this part connects each strand of the dsDNA when the hairpin is unfold, in our case we have a tetraloop (4 bases). This molecule is attached between two polystyrene microscopic beads flanked by two dsDNA handles at each side of the molecule. The aim of the handles is to prevent non-specific interactions (Fig 1-a).

To exert mechanical force to the molecule one bead is trapped in the optical trap, whereas the other bead is held fixed at the tip of a micro-pipette. With this set-up we can exert force on the molecule maintaining one bead fixed on the tip of the micro-pipette, and manipulating the other using the optical trap. Controlling the distance between the centre of the OT and the tip of the micro-pipette, distance λ, we can increase or decrease the exerted force on the molecule. To perform hopping experiments we maintain constant λ, while to perform experiments out of equilibrium we increase/decrease λ to change the exerted force. Then we obtain the elastic response of the molecule in a Force-Distance curve (FDC) (Fig. 1-b).

By fitting the stretching response (FDC) of DNA hairpin to a semi-flexible polymer model, equation 1, we have obtained the temperature dependence of the persistence length, L_p[3].

$$L_p = \frac{k_B T}{f_U}\left(\frac{4}{\left(1-\frac{x_{ssDNA}}{L_c}\right)^2}+\left(\frac{x_{ssDNA}}{L_c}\right)-4\right), \quad (1)$$

where f_U is the measured force when the molecule becomes unfolded. L_c is contour length, k_BT is the product between the temperature and the Boltzmann constant and finally $x_s sDNA$ is:

$$x_{ssDNA} = \frac{\Delta f}{k^F_{eff}} + d_0 \cdot \left(\coth\frac{f_U \cdot d_0}{k_B T} - \frac{k_B T}{f_U \cdot d_0}\right). \quad (2)$$

In equation 2 Δf is the difference of force at the moment when the stem breaks. k^F_{eff} is the slope of the FDC at moment of the molecule breaks in the branch when the molecule is folded. Finally d_0 is the diameter of the DNA hairpin when is folded.

Taking into account this elastic parameter we can characterize the free energy difference, ΔG_0, between the folded and unfolded state of the molecule. To calculate ΔG_0 we calculate de energy difference between both states performing hopping experiment (Fig. 1-c). To measure this we use the Kramer's theory that relates differences in energy whit the kinetic involved in each transition, Fig. 1-d.

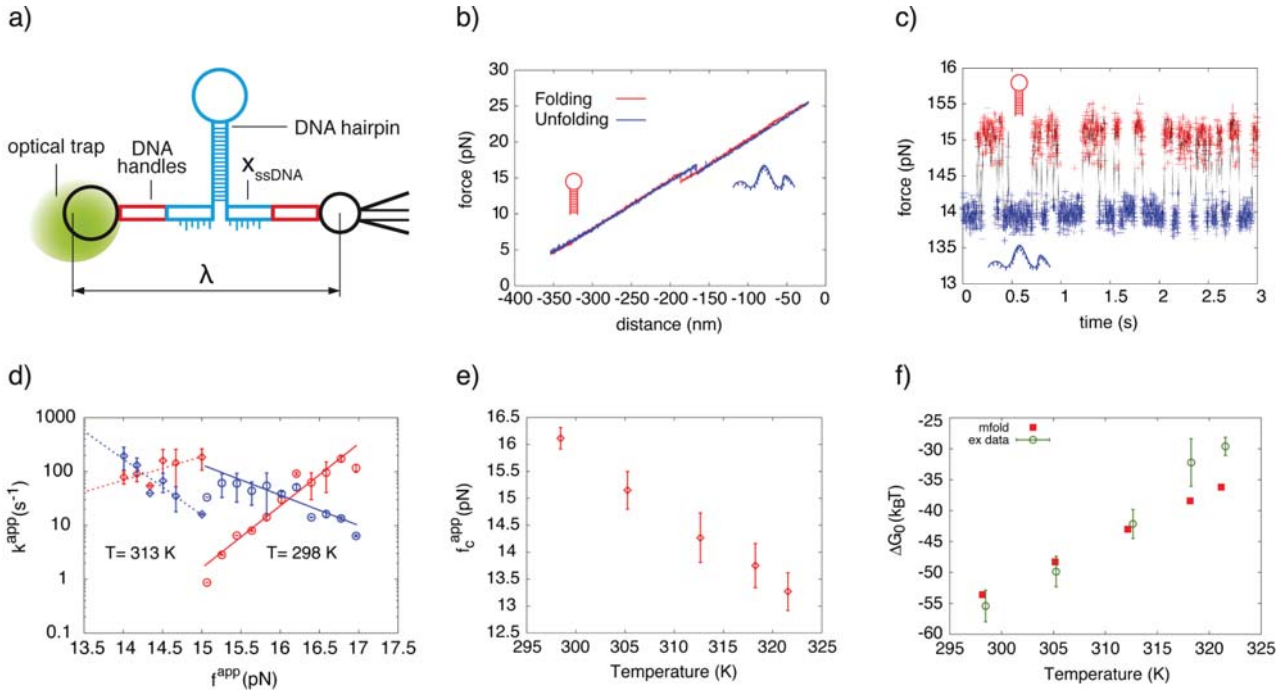

FIG. 1. a) The molecular construct is attached between two beads, one held by the suction of a micro-pipette and the other captured in the optical trap. b) Elastic response of a DNA hairpin presented in a Force-Distance curve (FDC). The rip in the FDC shows the moment when the molecule unfolds. c) Force-Time curve obtained during a hopping experiment. The blue points (high forces) are obtained when the molecules is folded while the red ones (low forces) are obtained when the molecule is unfolded. d) Kinetic rates at different force and temperature. The kinetics increase with temperature. The coexistence force is defined as the moment when both kinetics , unfolding and folding are equals. Lines (solid and dotted) are fits of equation 3 (blue) and 4 (red) e) Coexistence force as a function of temperature. The coexistence or the necessary force to open the hairpin decreases with temperature. f) ΔG_0 as a function of temperature.

$$k_{F\to U} = \frac{1}{\langle t_F \rangle} = k_0^* \exp\left[\beta\left(x_U \cdot f\right)\right] \quad (3)$$

$$k_{F\leftarrow U} = \frac{1}{\langle t_U \rangle} = k_0^* \exp\left[\beta\left(\Delta G - x_F \cdot f\right)\right] \quad (4)$$

In equations 3 and 4 k_0^* is the attempt rate, $\langle t_i \rangle$ is the average life time at each state, x_i is the distance between the minimum of potential for each state and the potential barrier. This barrier is the potential barrier that the molecule needs to overcome to change its state, $(i = F$ or $U)$. Finally ΔG is the energy difference between the folded and unfolded state. As we can see at a certain value of the force,f_c, both kinetic are equals, at this moment we say that the molecule are in coexistence, k_c.

$$\Delta G_0 = \Delta G - W_{handles} - W_{trap} - W_{ssDNA} - W_d \quad (5)$$

To find the free energy difference we need extract the contribution of each element to the energy difference (equation 5)[4]. W_{trap} is the work done by the OT, $W_{handles}$ is the work done by the handles, W_d is the work performed to orient de DNA hairpin when is folded and finally W_{ssDNA} is the work to stretch the ssDNA when the stem is unfolded.

In this report we present data obtained in a temperature range between 10 and 50 degrees. In Fig.1 are presented some significant results and also some plots to understand the main ideas behind this experiments.

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