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MASTER THESIS WORK

Thermodynamic analysis of a DNA hairpin using optical tweezers

Marc Rico Pastó

Supervised by Dr. Félix Ritort, (UB)
Dr. Marco Ribezzi-Crivellari, (UB)
Dr. Estela Martín, (UB)

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Thermodynamic analysis of a DNA hairpin using optical tweezers

M. Rico-Pasto[1]

[1] Facultat de Física, Departament Física Fonamental, Universitat de Barcelona, Av. Diagonal 647, 08028, Barcelona, Spain

E-mail: m.rico.pasto@gmail.com

Abstract. Since their invention 30 years ago, optical traps have emerged as a powerful tool in physics and biology. Capabilities of optical traps have rapidly evolved to study molecular and atomic systems. In this work we present an experimental optical tweezers setup capable of measuring forces and displacements, in the order of piconewtons and nanometers respectively. First, a brief description of the optical tweezers apparatus is presented. Next, we present some experiments performed with this setup aimed to extract the thermodynamic folding properties of a DNA hairpin molecule. Thus, the ability of optical tweezers to exert mechanical forces on molecular systems will be demonstrated.

Keywords: Optical tweezers, force measurement, DNA structure, thermodynamic properties, temperature dependence

1. Introduction

In the last decades a large number of astonishing molecular nanostructures and nanomachines 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 [2] to plastic micro-beads [1]. 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 [3, 4].

In this project we analyze the OT setup used in the Small BioSystems Lab of the University of Barcelona. We use this set-up to perform single molecule experiments and extract the thermodynamic and kinetic properties of a DNA hairpin under the effect of force and temperature. To conclude, we discus our results and compare them to existing literature.

2. Optical Tweezers

Arthur Ashkin pioneered the field of laser-based optical trapping in the early 1970s. In a series of seminal papers, he demonstrated that optical forces could displace and levitate microscopic dielectric particles in both, water and air. He also developed a stable three-dimensional trap based on counter-propagating laser beam [1]. Ashkin and co-workers employed optical trapping in a wide-ranging series of experiments from the cooling and trapping of neutral atoms [2] to the manipulation of living bacteria and viruses [5]. Since 1970s OTs have considerably evolved. It is now possible to control the position of OT (and hence the position of the trapped object) using, for example, piezo-electric actuators or acusto-optic devices. Furthermore, we are able to quantify the force exerted by the OT. The working mechanism of OTs can be understood via ray tracing scheme which takes into account the conservation of linear momentum upon light deflection (Fig. 1a).

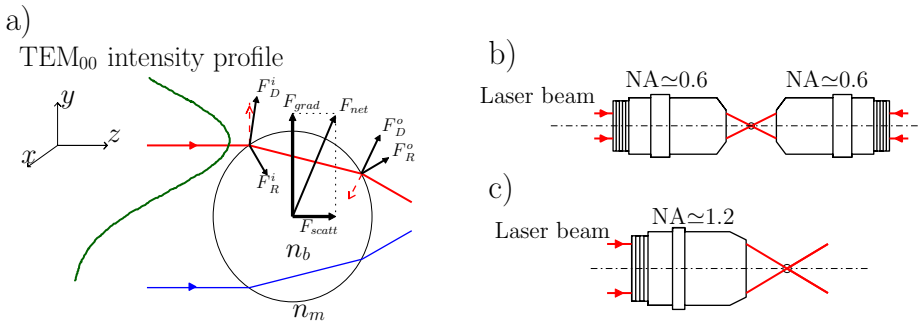

Figure 1. a) Ray tracing when a TEM_{00} passes through a dielectric bead. All rays are deflected (F_D) and reflected (F_R) at the two interfaces, in and output. The net force can be decomposed in two contributions, scattering force and gradient force. b) Two counter-propagating laser focused with two identical microscope objectives with numerical aperture around 0.6. c) One microscope objective with a high numerical aperture, around 1.2, focusing a laser beam.

In Fig. 1a we are assuming that the incident light comes from a TEM_{00} laser source, which has a Gaussian intensity profile. Also, rays that pass through a dielectric bead change their propagation direction due to reflection and refraction. Hence, knowing the refraction indexes of medium and bead, we can estimate the how much light is deflected and reflected by taking into account Snell's law. According to the law of linear momentum conservation, a change in the propagation direction of a ray implies the action of a force: $\overrightarrow{f} = \frac{\Delta \overrightarrow{P}}{\Delta t}$. This total force can be decomposed into two contributions. One is named **gradient force** (f_{grad}) and the other **scattering force** (f_{scatt}). The gradient force is the net force that pushes the bead to the region where the light is more intense, *i.e.* toward the center of the beam that has a Gaussian intensity profile, while the scattering force is a net force pushing the bead along the beam's propagation direction.

To create a stable three-dimensional trap A. Ashkin used two counter-propagating laser beams to obtain a space region where the scattering forces produced by the lasers cancel each other [1]. These two counter-propagating lasers are focused with two identical

objectives at the same point to obtain zero scattering force at the focal point (Fig. 1b). Nowadays we can use microscope objectives with high numerical aperture (NA) to improve the optical trap stability. For high NA, around 1.2, the focused cone of light is more pronounced (Fig. 1c) compared to the original set-up of A. Ashkin, and a gradient force appears in the direction of propagation. These kind of objectives can be used to create a stable optical trap with only one laser [6].

3. Experimental set-up

We will describe the OT setup presented in Fig. 2, which is in use in the Small BioSystems Lab at University of Barcelona [7, 8, 9]. It is a compact optical system in which the different elements, both mechanical and optical, are fixed to a structure which forms the device. This makes so that we are able to work with short optical distances, reducing the twinkling noise in the laser beam. The main feature of this devices is that it directly measure force via linear momentum conservation which guaranties a robust calibration (independent of the features of the trapped object or buffer medium).

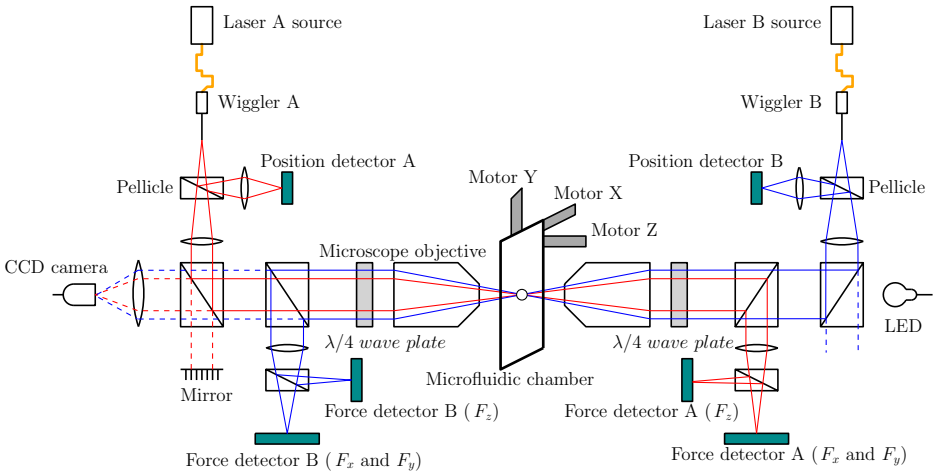

Figure 2. Optical system scheme to form an optical trap and measure directly the exerted force to the trapped object.

The setup presented in Figure 2 is a symmetric system, with two arms. Each arm has an identical microscope objective with high numerical aperture. In consequence, we can make two independent optical traps. For each trap we measure exerted force and trap position independently.

3.1. Force measurement

The salient feature of this instrument is that it directly measures the force exerted by the optical trap on the trapped object. To understand why it is important first we will explain how usual set-ups measure forces and the main drawbacks of this method. As we already said in section 2, the gradient force pushes the trapped bead towards to the center of the beam, where the rays are more intense. This force can be modeled

as a two-dimensional spring that exerts a restoring force in the (x, y) plane, which is perpendicular to the propagation direction. According to Hooke's law:

$$f = -k \cdot (x - x_0), \tag{1}$$

where f is the force exerted on the trapped object, k is the stiffness of the optical trap, x is the position of the bead with respect to the center of the optical trap and x_0 is the position of the center of the trap.

Once the stiffness of the trap is known, taking into account the Eq. (1), we can measure forces by determining the position of the bead. However, the stiffness, k, depends on the experimental conditions, *i.e.* the medium used to perform experiments, the material, shape and size of the beads and laser power. Consequently, the OT must be calibrated every time an experiment is performed. Moreover, Eq. (1) is only a linear approximation and non-linearities can appear at high forces. This drawbacks are solved by direct force measurement based on linear momentum conservation which provides a very robust approach independent of the experimental conditions (Fig. 3a).

A transparent particle of high refractive index (higher than that of the medium) is trapped at the focal point by virtue of the way it deflects photons and changes their linear momentum. If the trapped object is somewhat offset from its equilibrium trap position (Fig. 3b), at the light focus, by an external force f, then the flux of light exiting the back side of the trapped bead will undergo a change in momentum equal to:

$$f = \sum_i \frac{dP_i}{dt}, \tag{2}$$

where the summation runs over all rays entering (and leaving) the trap. By determining the changes in the angular power distribution of the rays coming out at the back side of the trap, it is possible to measure the external force applied on any trapped object (Fig. 3b). Consequently, we measure forces measuring changes in the pattern light in the detector. If we apply an external force, for example by flowing buffer, we offset the bead position from its equilibrium trap position (where $f = 0$). The output light beam is projected by relay lenses and split by non-polarizing beam splitter cubes into two beams that reach two separate detectors that measure the transverse forces (f_x and f_y) and the axial force (f_z) separately (Fig. 3c). Hence, we can quantitatively measure forces not only in the plane perpendicular to the propagation direction, but also in the parallel direction. This method directly measures forces and does not require of any approximation.

This approach to determine forces requires that the total amount of deflected light is collected in the detector, independently of the position of the trapped bead, because the the sum on Eq. (2) includes the condition of all deflected rays. For this reason we work with an underfilling condition (Fig. 3d). It means that the waist of the collimated laser beam that we focus cannot fill all the entrance pupil, to be able to redirect all the rays at any exerted force. Consequently, we work with an effective numerical aperture around 0.6, which is a low NA in comparison with the real numerical aperture of the microscope objective. As we want to have a three dimensional stable optical trap we focus both lasers at the same point, as in the original set-up proposed by A. Ashkin

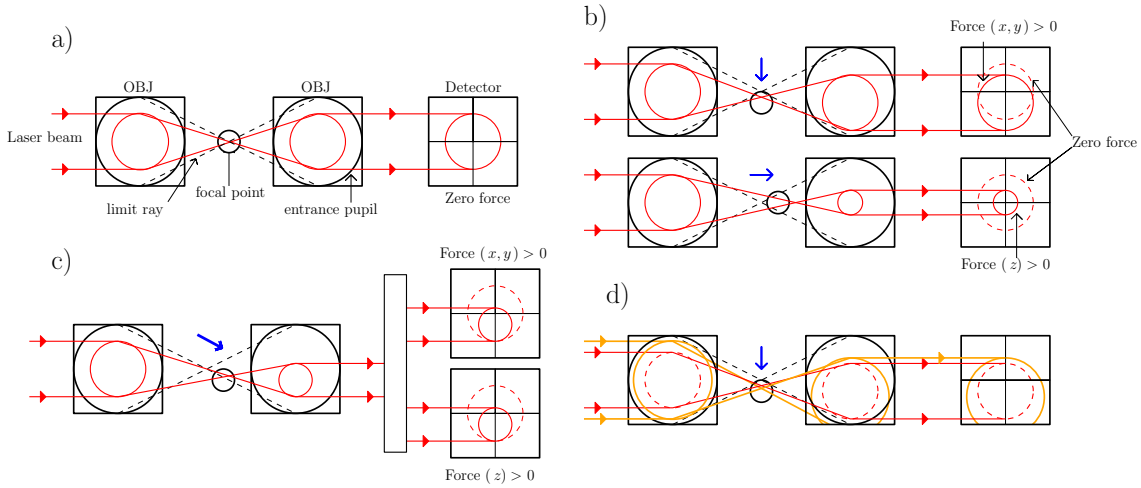

Figure 3. Light patterns when we apply an external force. a) Zero force: Laser beam enters through objective lens (OBJ) and traps bead at the focus. Beam then passes out the back side, is collimated by the 2nd objective and projected onto detector surface. b) Force from above (left) represented by the blue arrow pushes light beam downward (pushing bead away from laser source). Pattern is offset down (spot contracts in the detector). c) Force pushes the bead away from the laser source and down. d) Laser beam that covers all the entrance pupil (orange beam). When the bead is somewhat offset from its equilibrium position part of light is missed in the detector.

(section 2). Only with silica beads (that has a refraction index closer to the index of the water) we obtain two stable individual traps [10].

4. Single molecule experiments with OT

In our original setup we use the optical trap to manipulate single molecules. The setup is described in Fig. 4. It consists of a molecule attached between two polystyrene microscopic beads. The molecule is flanked by two dsDNA handles at each side of the molecule. The aim of the handles is to prevent non specific interactions. One bead is trapped in the optical trap and is used as a force probe, whereas the other bead is held fixed at the tip of a micro-pipette. With this setup 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 (Fig. 4). In this set-up the control parameter is the distance between the micro-pipette and the center of the optical trap, λ. λ should not be confused with the molecular extension. In fact when we exert force the trapped bead is displaced from the center of the trap a distance x. The length of the molecule L is related to λ and x by:

$$L = \lambda - x \tag{3}$$

We can use different molecules, from DNA to ribonucleic acids (RNA) or proteins and study their thermodynamic and elastic properties.
In this project we work with a DNA hairpin structure. These are self-complementary single stranded DNA (ssDNA) fragments which in solution will fold onto themselves. When DNA hairpins are folded we can differentiate two parts: the stem and loop. The

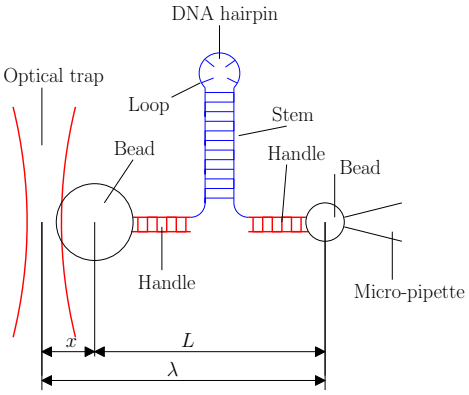

Figure 4. Microscopic configuration to perform experiments. One bead is trapped by the optical trap while the other is fixed in the tip of a micro-pipette. λ is the distance between the optical trap and the tip of the micro-pipette, x is the distance between the center of the trap and the center of the trapped bead and L is the molecular extension.

stem is formed by the part of ssDNA that can form base pairs, producing a dsDNA fragment. Synthetic DNA hairpins can be easily designed to have different stem (and loop) length and sequence.

4.1. Equilibrium - Hopping experiments

With the described setup (Fig. 4) we can perform different kinds of experiments to study the thermodynamic and elastic parameters. In this report we will focus on named **hopping experiments** which use short DNA hairpins to study the molecule's behavior under force. In our case we use a DNA hairpin with 20 base pairs (bp) in the stem and a tetraloop (Fig. 4).

We trap a DNA hairpin by keeping constant the distance between the optical trap and the tip of the micro-pipette, *i.e.* λ is constant during the experiment. When held under sufficient force the hairpin can spontaneously switch back and forth between the folded state, due to thermal fluctuations. When the molecule is unfolded it has a molecular extension around 40 nm (20 nm of the handles plus 20 nm of the unfolded ssDNA), while when it is closed (folded state) the molecular extension is around 22 nm. When the molecule changes extension at constant λ the distance x changes too leading to a force jump. During the hopping experiment we will observe two levels of force, one when the molecule is folded and other when it is unfolded (Fig. 5a).

The free molecular energy landscape ($V^0(x)$) of these processes are typically modeled as two potential wells separated by one barrier (Fig. 5b). We define each state (folded and unfolded) as the local minimum of these potential wells. Furthermore the position of the barrier is defined as the position of the local maximum ($x^\dagger$). We can define the distance between the barrier and the position where the molecule is folded as x_F and the distance between the barrier and the position where is unfolded as x_U (Fig. 5b). Consequently, we define the change in molecular extension from folded to the unfolded states as $x_m = x_F + x_U$.

The kinetic rates, defined as as the inverse of the life time of each state (unfolded and folded), can be modeled taking into account Kramer's theory. This theory relates the kinetic rate with $V^0(x)$ as:

$$k_{F\to U} = k_0 \cdot \exp\left[\beta\left(V^0(x_F) - V^0(x^\dagger)\right)\right], \tag{4}$$

$$k_{F\leftarrow U} = k_0 \cdot \exp\left[\beta\left(V^0(x_U) - V^0(x^\dagger)\right)\right], \tag{5}$$

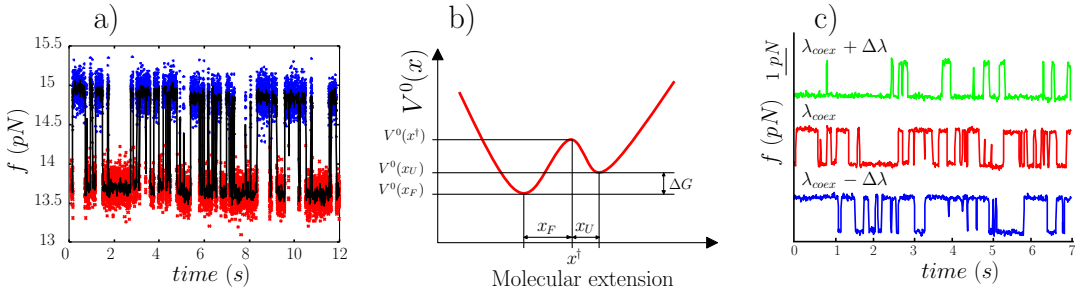

Figure 5. a) Force-time trace at a certain λ. We can differentiate clearly two states: folded state represented by blue points (high value of force) and the unfolded state represented by red points (low force). b) Free energy landscape of a system with two states. c) Force time curves obtained at different values of λ. The green curve (upper curve) is obtained when we favor the unfolded state, the red (central curve) we have coexistence and blue (bottom) curve when the folded state is favored.

where k_0 is kinetic attempt rate and $\beta = \frac{1}{k_B T}$ where k_B is the Boltzmann constant and T is the absolute temperature. Under an externally applied force, f, the free energy landscape changes by $V_f(x) \to V^0(x) - fx$. Leading to:

$$k_{F\to U} = k_0 \cdot \exp\left[\beta\left(V^0(x_F) - V^0(x^\dagger) - f \cdot x_F\right)\right], \tag{6}$$

$$k_{F\leftarrow U} = k_0 \cdot \exp\left[\beta\left(V^0(x_U) - V^0(x^\dagger) - f \cdot x_U\right)\right]. \tag{7}$$

Which can be rewritten as:

$$k_{F\to U} = k_0^* \cdot \exp\left[\beta\left(x_U \cdot f\right)\right] \tag{8}$$

$$k_{F\leftarrow U} = k_0^* \cdot \exp\left[\beta\left(\Delta G - x_F \cdot f\right)\right] \tag{9}$$

where $\Delta G = V^0(x_F) - V^0(x_U)$ is a thermodynamic parameter that defines the energy necessary to break the hydrogen bonds which form the dsDNA. These kinetic rates satisfy the detailed balance condition which relate them with the probability to find the molecule in each state by:

$$\frac{P_F}{P_U} = \exp\left[\beta((x_U + x_F)f - \Delta G)\right] = \frac{k_{F\leftarrow U}}{k_{F\to U}}. \tag{10}$$

Taking into account this discussion we can see that changing the external force we can favor each state (Fig. 5c). *i.e.* increasing (decreasing) λ we favor the unfolded (folded) state (upper and lower plots in Fig 5c).
We define the coexistence force, as the force where $k_{F\to U} = k_{F\leftarrow U}$ or $P_U = P_F = 1/2$, (red curve in Fig. 5c).
Equations (8 - 10) depend on the temperature. In this report, we will investigate the folding/unfolding kinetics in a temperature range from 25^{o}C to 50^{o}C.

5. Experimental results

Experimentally we can analyze the temperature dependence of both ΔG and x_m. To analyze these quantities we can follow two strategies. First we can use the detailed balance condition (Eq. (10)) and second we can fit the kinetic rates values to equations (8) and (9).
In Fig. 6a we present the behavior of the molecule when we change temperature. The molecule starts from coexistence and passes to be completely opened upon a change in temperature (arrow in Fig. (6)). After approximately 15 seconds we change λ to lower the force and allow the molecule to fold back (marked with *).

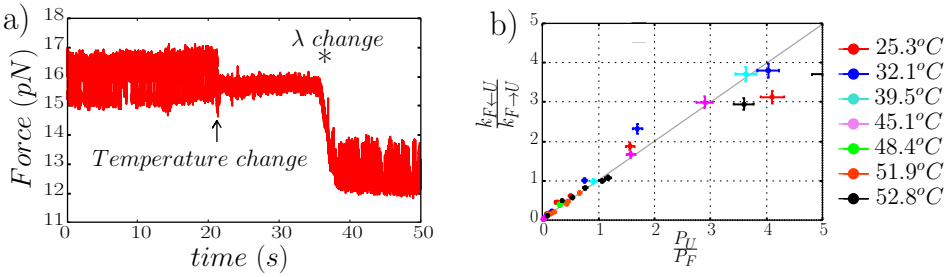

Figure 6. a) Force time trace obtained when we change the temperature. The arrow represents the moment when we change the temperature of the chamber. The moment when the molecule folds by changing λ is marked with *. b) Relationship between probabilities ratio and the kinetic rates ratio. It follows a linear expression with slope equal to one. Color represent different temperature, same as in Fig 7a, (Eq. (10)).

First we use the detailed balance condition. To do this, we compare the probability ratio ($\frac{P_U}{P_F}$) and kinetic rates ratio ($\frac{k_{F\leftarrow U}}{k_{F\rightarrow U}}$) at different temperatures, Eq. (10). If the detailed balance condition is fulfilled these quantities ($\frac{P_U}{P_F}$ and $\frac{k_{F\leftarrow U}}{k_{F\rightarrow U}}$) must be equal (Fig. 6b).
To obtain ΔG and x_m we represent the probability ratio or the kinetic rates ratio as a function the measured force (Fig. 7a and b). Fitting the probability ratio with Eq. (10) we can find the temperature dependence of ΔG (Fig. 7c) and x_m (Fig. 7d), represented by the red points in both figures. Nevertheless, these are not the unique parameter that we can find, we can find also the coexistence force, f_c, at different temperature when $P_U = P_F$. In consequence is possible to find the coexistence force taking into account this condition. An approximated value of the coexistence force is indicated with dashed lines in Fig. 7a and b.
On the other hand, we can study how kinetic rates change when we change both, the distance λ and temperature (Fig. 7e). In this case to study ΔG and x_m we $k_{F\leftarrow U}$ to the equation (8) and $k_{F\rightarrow U}$ to equation (9). Notice that we are assuming that k_0^* does not depend with the force. The results for ΔG and x_m are shown in Fig. 7c and d with blue points. The coexistence force, f_c, are marked with dashed lines in Fig. 7e when $k_{F\leftarrow U} = k_{F\rightarrow U} = k^c$. We can also measure how the kinetic rate at coexistence, k^c, changes as a function of temperature (Fig. 7f).
To conclude we will compare our results using both strategies. First we will compare our results of ΔG and x_m as obtained: 1) tacking into account the detailed balance condition and 2) using equations (8) and (9) (Fig. 7a and b). As we can see in these figures both

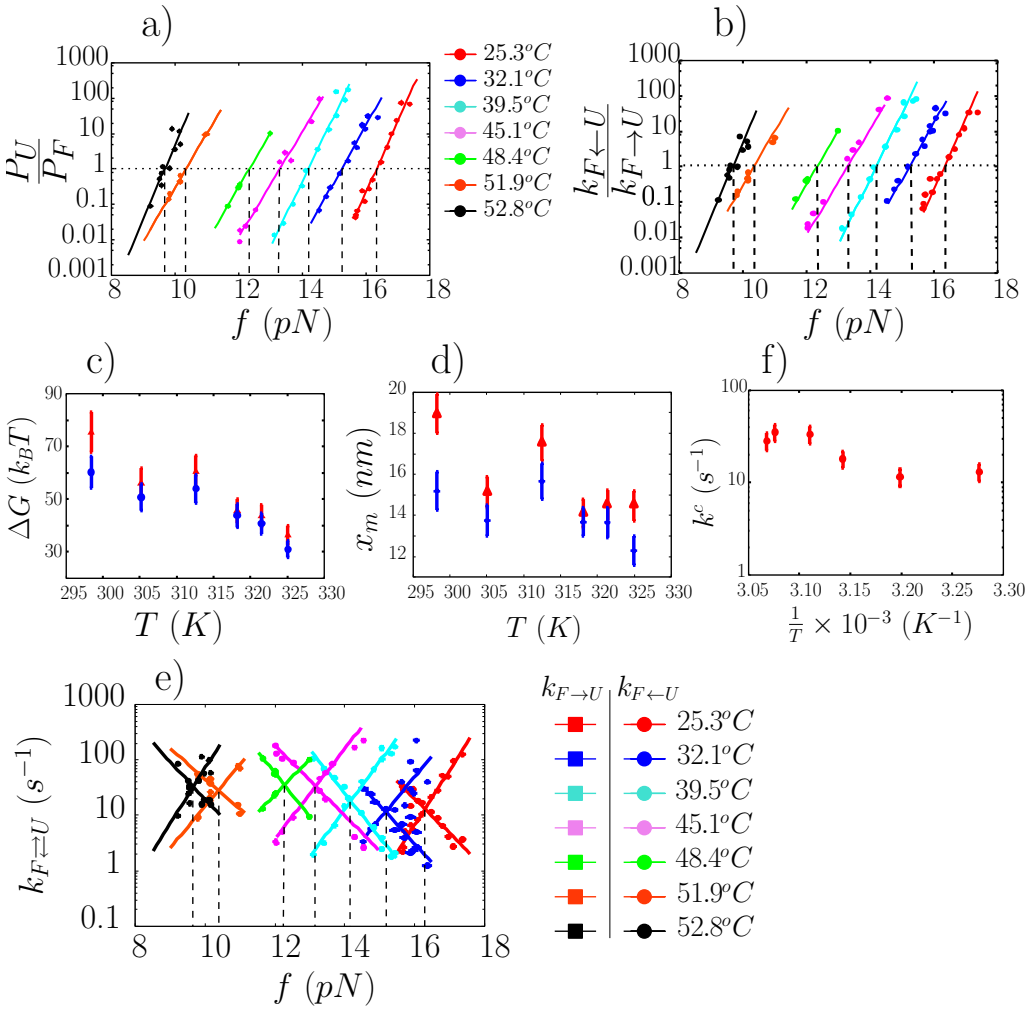

Figure 7. a) Probabilities ratio as function of force at different temperatures. b) Kinetic rates ratio as function of force at different temperature. c) ΔG as function of temperature. d) x_m as function of temperature. e) Kinetic rates as function of force at different temperature. f) Kinetic rate at coexistence as function of temperature.

results coincide with an average error around 7%, it means that both strategies are valid to study it.

6. Conclusions

Our results can be compared with existing literature [11, 12] (Fig. 8a and b). First, we will compare our results of x_m/L_p (where L_p is the contour length) as a function of force with the results of [11]. In [11] they perform another kind of experiment to study the force dependency of x_m. As we can see in Fig. 8a our results are coincide. Notice that we do not directly present the molecular extension (x_m) as function of force because in [11] they study different lengths. Second, we compare the coexistence force as function of temperature with the results of [12]. In [12] a pulling experiment is performed to obtain the coexistence force at different temperature. As we can see in Fig. 8b our results are agree with the results presented in [12] throughout all the temperature range.

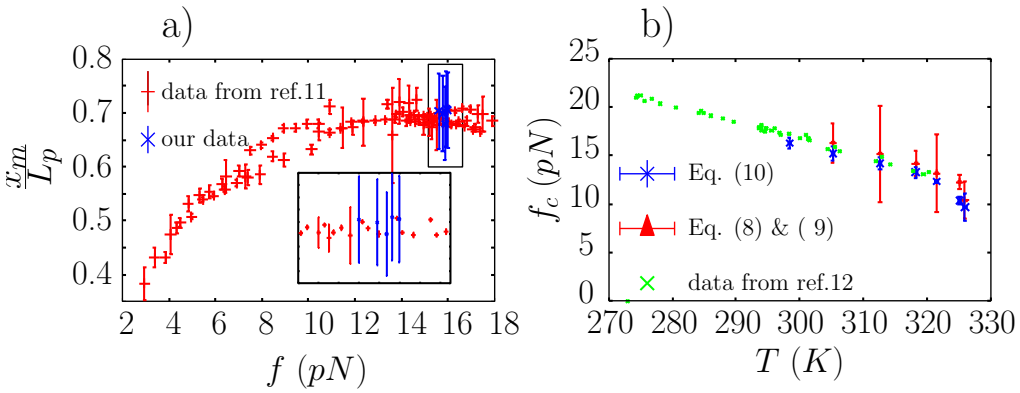

Figure 8. a) Relationship between x_m/L_p and the exerted force. Red points are the obtained data in [11] while the blue points are our obtained data. b) Coexistence force at different temperature.

Currently we are working toward increasing the temperature range to prove if our experiments are agree with literature in all temperature range.

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