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Master in Photonics

MASTER THESIS WORK

**Biochemical noise in single DNA
molecule studied by optical trapping
technique**

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Presented on date 9th September 2010

Registered at

 Escola Tècnica Superior
d'Enginyeria de Telecomunicació de Barcelona

Biochemical noise in a single DNA molecule studied by optical trapping technique.

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Abstract. Noise spectra from single DNA molecules in their natural aqueous environment at different grades of stretching are presented. A DNA molecule is anchored between two dielectric beads. One of the beads is fixed to the surface and the other is optically trapped. The stiffness of each stage is calculated and compared with the previous results to verify the presence of one and only one single molecule. The results are compared with the preceding bibliography and are focused in the study of the spectral response which is not deeply studied yet.

Keywords: noise, optical trapping, DNA, WLC model

1. Introduction

DNA molecule has a width of approximately 20 Angstroms and it can be as long as 11 cm in some chromosomes. This molecule is inside the cell nuclei which is around few microns in size. Important point here is the order of magnitude difference between length of cell nuclei and total extension of DNA. DNA has to rearrange itself in a reduce size. Moreover, the tertiary structure of DNA must be broken so that important phenomenon related with DNA (translation, translation or replication) can occur. Is the physics of the system the same in all different structures? How can DNA reduce many times its size and don't lose its properties? This master thesis is the first step to analyze possible noise assisted processes in DNA. Here, DNA is manipulated using optical trapping technique which allows to apply a force of the order of magnitude (pN) needed. Several models to understand DNA have been proposed. Treating DNA as a polymer, worm-like-chain model is able to explain changes in the stiffness of DNA, but it is unable to explicate different power spectrum densities (PSD) that are shown in this research.

1.1. Optical tweezers technique

Optical trapping technique was developed in the late seventies by Arthur Ashkin [1]. A laser beam is highly focused by a high NA objective creating an electrical field gradient inside a dielectric object. This gradient force is also added to the radiation pressure of photons. These two forces make an almost symmetric potential in transverse direction of the path, and a non symmetrical one in the parallel axis. These forces are in the range of pN and allow to hold and to manipulate micro size object. Around the equilibrium position it is possible to approximate this system to a spring which facilitates the calibration of tweezers. Stiffness of the trap is not only dependent on the tweezers setup, but also depends on geometry of the bead, dielectric index, size, viscosity of the media, temperature of the sample, etc.... Also metallic beads can be trapped, but a different physics involves that. Dielectric beads were used in this research. Optical tweezers is particularly a great tool in biophysics, enabling to study from cells or bacteria [2, 3] to molecular motors in real time, allowing to manipulate biological molecules at singular level and giving us precious information to understand the laws that are ruling this micro sized world.

1.2. DNA. Worm-like-chain model.

DNA is a nucleic acid which contains all the necessary instructions for known life [4]. It composes of four bases and organizes in different structures, fig. 1.1. DNA is a double-stranded molecule. In its three dimensional structure it is possible to distinguish different levels:

- Primary structure: DNA is formed by four different bases (A,C,G,T). This structure is the sequence of this different basis coding the information to produce all the different proteins. It is in this order where hold all difference in life.
- Secondary structure: This is the famous double helix structure. This helps in explaining the genetic information storage and DNA replication mechanism. Chains are complementary, adenine must join with thymine and guanine must join to cytosine. Left-handed or right handed nature of these helices depends on DNA type.
- Tertiary structure: This structure allows a long molecule like DNA in a small place like cell nuclei. To achieve that, DNA takes the form of chromosomes. This form depends on the organism, eukaryotes or prokaryotes. In prokaryotes DNA folds like a super-helix, usually of circular topology, and associated to an amount of proteins. In eukaryotes, information is higher, then DNA is much longer and chromosomes are bigger. It needs a more efficient packet, more complex and compact. Now proteins are needed, like histones, protamines, etc...



It is in this tertiary structure where we are working, trying to model different response at different forces. Different model are proposed depending on different force regime. In this work forces from 0.5 pN to 5pN are used, and Worm-Like-Chain model (WLC) provides an excellent approach to this regime [5]. This is a model of long chain polymer which can be applied particularly to DNA. Briefly, like any polymer in solution, DNA takes a random conformation which maximizes its entropy. The entropic force tries to leave a state to another with more possible configurations. This type of forces rules up to 10 pN, typical range of our experiment. Forces higher than 10 pN and up to 70 pN make DNA acts like a spring, this is called enthalpic regime.

Fig. 1.1. Schematic picture of DNA structures. From left to right: Primary structure based in covalent bounds between nucleotids. Secondary structure which is the characteristic double helix. Tertiary structure, divided in different steps from histones to chromosome shape.

In this entropic forces regime, it is possible to apply the Worm-Like-Chain (WLC) model, not a very complicate vision of polymer. Consider the polymer as a chain with no torsional stress, a succession of N segments of length b, each one with an orientation \mathbf{t}_i . Like in a Pot model, the total energy of the system is described by the sum of the bending energies:

$$\mathcal{E}_{KP} = -\frac{B}{b} \sum_{i=2}^N \vec{t}_i \vec{t}_{i-1} = -\frac{B}{b} \sum_{i=2}^N \cos \theta_i \quad (1.1)$$

where B is the bending modulus and θ_i is the angle between two adjacent segments. Here it is possible to probe that the angular correlation decays exponentially with the distance:

$$\langle \vec{t}_i \vec{t}_{i-1} \rangle = e^{-b|i-j|/\xi_T} \quad (1.2)$$

being $\xi_T = B/kT$ the decay length of the angular correlation, also known as persistence length. Next step is to include a tension inside the model to add possible effects of stretching. In eq. 1.1 a work term is added, $W = -\vec{F} \vec{R} = -Fb \sum_{i=1}^N t_{i,z} = -Fb \sum_{i=1}^N \cos \Theta$, where force is applied in z-axis, then Θ is the angle between every orientation vector of any segment with z-axis:

$$\mathcal{E}_{KP} = -\frac{B}{b} \sum_{i=2}^N \vec{t}_i \vec{t}_{i-1} - Fb \sum_{i=1}^N t_{i,z} = -\frac{B}{b} \sum_{i=2}^N \cos \theta_i - Fb \sum_{i=1}^N \cos \Theta_i \quad (1.3)$$

Until here, DNA was described by the Kratky-Porod model. Problem with this model is that it is unsolvable for not very small forces. One of this approximation is afforded by the WLC which take the continuous limit of eq. 1.3, segment length (b) tends to zero.

$$\frac{\epsilon_{WLC}}{kT} = \frac{\xi_T}{2} \int_0^{l_0} \left(\frac{d\vec{t}}{ds} \right)^2 ds - \frac{F}{kT} \int_0^{l_0} \cos \Theta(s) ds \quad (1.4)$$

where s is the curvilinear coordinate along the chain. Calculus of the partition function and the free energy of the system are quite close to the quantum mechanical problem of a dipole in an electric field. Though no analytical formula is achieved, several numerical approximation have been done [6], in this work we will use the numerical solution of Bustamante *et al.*, [5]:

$$\frac{FP}{kT} = \frac{1}{4} \frac{1}{\left(1 - \frac{x}{L}\right)^2} - \frac{1}{4} + \frac{x}{L} \quad (1.5)$$

where L is the molecular contour length and P is the persistence length.

1.3. Noise. Importance of characterization in DNA

Noise is defined as a stochastic signal around some deterministic value. Although noise has this random nature, it is possible to define statistical behaviors and properties. Different types of noise can be defined by the study of its power spectral density (PSD). These different types of noise are defined as the different colors of noise. This terminology is a parallelism between wavelength of visible spectra and the acoustic spectra [7]. One well known color of the noise is the white one, also known as Brownian motion, which has uniform distribution of energy in all frequencies.

Noise appears in many different places in nature, from heart rate to economy, passing through motion of molecular motors [7]. This stochastic performance of this motors are thermodynamics fluctuation directly linked with noise. In optical trapping, white noise is included by means of the micro sized bead trapped in suspension. This diffusion process is translated to white noise spectra, but only at low frequency since at high frequencies viscosity effect starts to dominate, it is translated as a -2 slope.

One hypothesis of this noise source is that DNA is storing energy which come from Brownian motion continuously, but when DNA is stretched the conformation of the polymer change, consequently the way of store and give off this energy has to change, making frequency an important factor of study, and as our results show, dependence in frequency increases with stretching. The importance of this noise comes from it possible combination with well studied energy sources of molecular motors [8] being a way to take energy of Brownian motion from cytoplasm (or whatever can be the source) and use it in transcription, replication and translation processes.

2. Experimental and methods

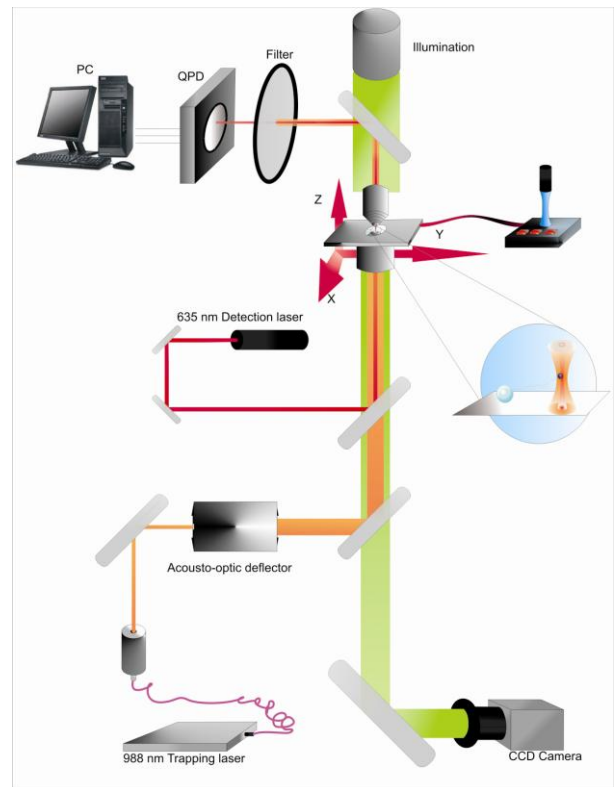


Fig. 2.1. Simplified optical layout for the DNA binding. Trapping laser goes across an acousto-optic deflector whose crystal is conjugated with the objective by two lenses in order to be able to move very precise the trap in one axis. These two lenses also expand the beam to better focusing. Position detection laser path is composed by two mirror with the goal to find the best interference pattern as possible. After objective and collimator, a mirror reflects trapping and detection laser, so it is necessary one filter to avoid the arrival of infrared light into the QPD. In this setup, it is used a LP 700 filter. Movement of chamber is made by a piezostage system.

2.1. Experimental setup

Experimental setup of optical tweezers is represented in Fig. 2.1. This setup has been used in our previous experiment. Briefly, optical trap was made with a diode laser of 988 nm with a power around 20 mW. Laser is focused by a 100X oil immersion objective (Nikon, 1.30 NA). Position detection is made with a HeNe laser of 635 nm wavelength, average power of 0.2 mW, and a large area quadrant photodetector (QPD, Model 2911, New Focus). This QPD has a good response below an acquisition frequency of 75 kHz. All the data were registered with 50 kHz. Change from electrical signal of QPD to digital is done by a shielded connector block with BNC for X Series and M Series (National Instruments BCN-2110). Control of the chamber and objective is done by a piezostage system (model NV40/3s, JENA) which allows us to explore an area around 1 mm² with a precision around 5 microns. Trapping laser is modulated in stretching direction with an acousto-optic deflector (AOD LS55, ISOMET). Finally, illumination was made by a green LED.

2.2. Sample preparation and DNA binding

DNA was amplified using a standard PCR process and immediately frozen. The molecule used was a double stranded λ -DNA 12 kbp from *E. Coli*. In the PCR process Biotin molecule was fixed to one extreme of the DNA molecule, while in the other end digoxigenin is set. Bovine serum albumine (BSA, 0.1 mg/ml) and phosphate buffered saline (PBS, pH ~7.4) in deionised water were mixed to produce the buffer

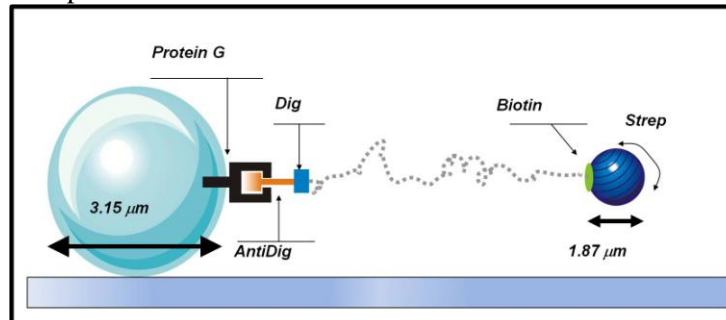


Fig. 2.2. DNA attached to two beads. Big particle, 3.15 microns, is covered by protein G and stuck to the coverslip. Small bead, bathed in Streptavidin, is bonded to the other end of DNA

to dilute the sample. DNA binding is divided in two parts. First, DNA is bonded to polystyrene bead bathed in Streptavidin (STREP, diameter 1.87 μm. G. Kisker GbR) by the biotin. Other solution is prepared to fix the other end of DNA. There, polystyrene bead with Protein G in its surface (DIG, diameter 3.15 μm. G. Kisker GbR) are mixed with Anti-digoxigenin (ANTIDIG, Roche Diagnostics GnbH) which reacts with Protein G, in this case it's necessary two chemical products to fix both extremes of DNA, 3' and 5'. In the other case we can only use STREP because it produces a blunt end, a 3' overhang or a 5' overhang. When both solutions are ready, they are mixed and injected to the custom flow chamber. Fig. 2.2. gives us a brief idea as how attachment is done.

2.3. Methodology

Next step is to find one DIG bead stuck, non-specifically, to the surface and approach with a trapped STREP bead near it. When the beads were in contact for around thirty seconds the DIG-ANTIDIG chemical binding attached the free end of DNA with a high probability, close to 75%. As the DIG bead is banished from the trap position, the DNA is stretched and the STREP bead is subjected to a restored force, enabling view if the DNA binding has been successful. A schematic picture can be seen in Fig. 2.3, where it is possible to see the final arrangement. The aim of this work is to study a single DNA molecule; therefore it is necessary to check the binding is produced by one and only one molecule. It can be done by fitting the extension curve to the WLC model. Fig. 3.5 contains the information of one measurement. Before start, register the position of the bead, it is necessary to check the interference pattern of the detection laser to be in the linear range of the procedure. In this case, trapping laser is moved with an acousto-optic deflector in the same sense as the stretching with the aim of putting the bead in the middle of the

detection laser. Once DNA is attached it is necessary to stretch as long as possible. Total stretching motion is not continuous; DNA had several knots along itself which has to be untied. Waiting few seconds before starting the measurement is enough to see several small jumps in bead position (this moment is also useful to see if DNA was stretched only in one axis, then X and Y wouldn't be mix). After that, we placed the place in the edge of the linear range of the detector, realizing that no motion of the bead went out of this range. Immediately, measurement no longer than 10 seconds were taken for different stretching until the opposite side of the linear range is achieve, fig 3.2. This is repeated trying to achieve each time more stretching until DNA broke. When it occurs, with the same bead, but this time free, one 100s calibration measure is made to know the trap stiffness.

3. Results

3.1. Calibration of the optical trapping system.

There are several methods in order to calibrate an optical tweezers [9]. It is possible to use from the time of flight of the particle to the dynamics of the bead in a constant flow. In this work we are going to use the Brownian motion of the bead in the trap. This random motion is in the order of magnitude of the optical trapping forces, and it is possible to write the force balance, eq. (3.1), with the assumption that the bead is in the linear range of the trap:

$$m\ddot{x}(t) + \gamma\dot{x}(t) + \kappa x(t) = \sqrt{2kT\gamma}\eta(t) \quad (3.1)$$

where m is the mass of the bead, $\gamma = 6\pi R\mu$ is the friction coefficient (being μ the viscosity and R the bead's radius) always supposing that we are working in the Stoke's regime, κ is the trapping constant, k the Boltzmann constant, T the temperature and $\eta(t)$ the stochastic function. This random function must be Markovian and it has to respect that mean value is zero, $\langle\eta(t)\rangle = 0$, and its

autocorrelation is a Dirac delta, $\langle\eta(t)\eta(t')\rangle = \delta(t - t')$. Then, force balance goes to Fourier space, neglecting the inertial term, and power spectrum density of position is calculated [EQ]:

$$-i2\pi f\gamma\tilde{x}(f) + \kappa\tilde{x}(f) = \sqrt{2kT\gamma}\tilde{\eta}(f) \quad (3.2)$$

$$PSD(f) = |\tilde{x}(f)|^2 = \frac{kT|\tilde{\eta}(f)|^2}{2\pi^2\gamma(f^2 + f_c^2)} \quad (3.3)$$

where we applied white noise nature of $\tilde{\eta}(f)$ and allivated notation by definition of the corner frequency (f_c), $|\tilde{\eta}(f)|^2 = 1$ and $f_c \equiv \frac{\kappa}{2\pi\gamma}$

It is useful to remember that the goal of calibration is to know the constant of the trap and the conversion factor from arbitrary units to meters. Now, both unknowns can be solved by fitting a Lorentzian curve to the experimental data. Trapping constant is estimated through the corner frequency. The conversion factor is calculated comparing the value of kT/γ in the Lorentzian fit with the theoretical values. Fig. 3.1. represents the experimental points and its fitting.

Then, trap stiffness is the following: $f_c = 32.2\text{Hz} \Rightarrow \kappa = 3.17 \frac{\text{pN}}{\mu\text{m}}$

and the conversion factor: $\beta \left(\frac{\text{m}}{\text{a.u.}} \right) = \sqrt{\frac{A_{\text{theoretical}}}{A_{\text{fitting}}}} = 1.04 \cdot 10^{-7} \frac{\text{m}}{\text{a.u.}}$

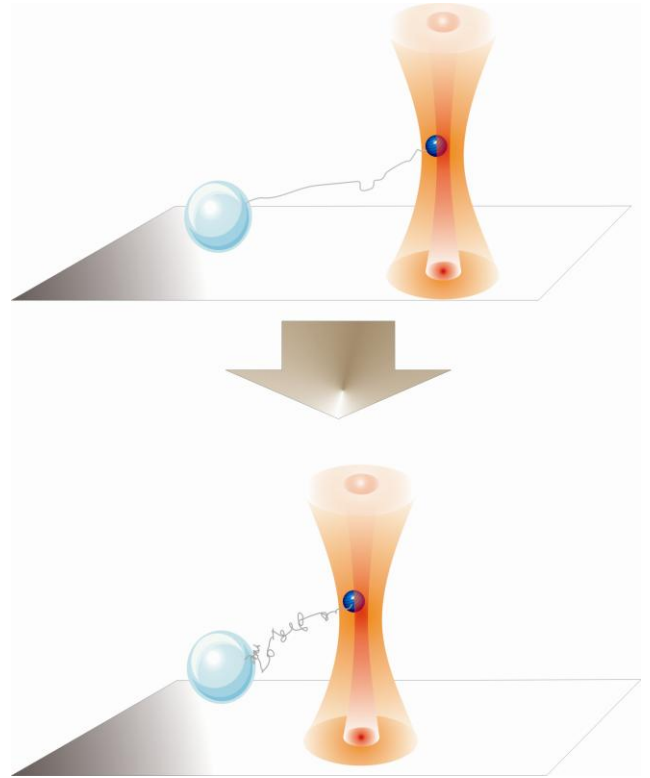


Fig. 2.3. Schematic vision of stretching. Small bead represents STREP, big one DIG. Red and Orange beams represent detection and trapping laser severally. Upper one corresponds to completely Stretched: All knots are vanished and STREP bead is strongly pushed out of the trap. Lower one correspond to last position recorded: Molecule is not tighten as long as possible and many knots are already tied.

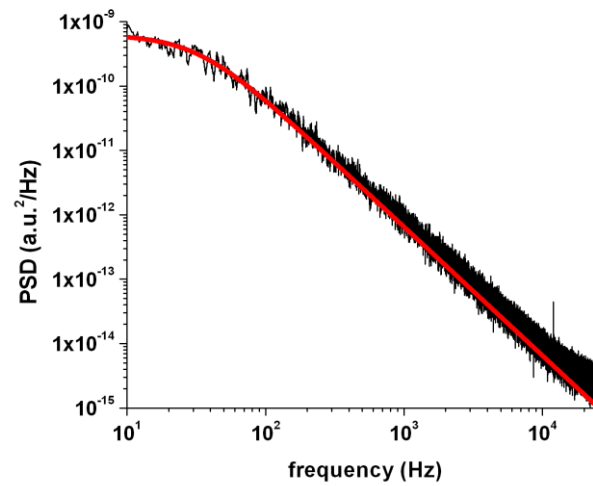


Fig. 3.1. Power spectrum density of the free bead (black). Red line plots the fitting. $PSD(f) = \frac{A}{2\pi^2} \frac{1}{f^2 + f_c^2}$; $A_{fitting} = (1.2870 \pm 0.002) \cdot 10^{-5} a. u.^2 Hz$; $f_c = (32.17 \pm 0.02) Hz$

3.2. Stiffness of DNA molecule. Comparison with WLC model.

One time the calibration of our optical tweezers is done, position of stretched DNA experiment are recalculated in meters. In fig.3.2. the trajectory is plotted versus time, each colour correspond to a different stretching. Correspondences between legend and distance are written in table 1. It is necessary to note that the distance wrote is the difference between the mean values of St1 with the mean value of the St noted.

Table 1. Correspondence between legend and distances. Distance are calculated from the most stretched case (St1).

	<i>Distance relaxed from St1(nm)</i>
St1	0±17
St2	90±17
St3	213±19
St4	250±21
St5	288±23
St6	323±24
St7	352±29
St8	373±27

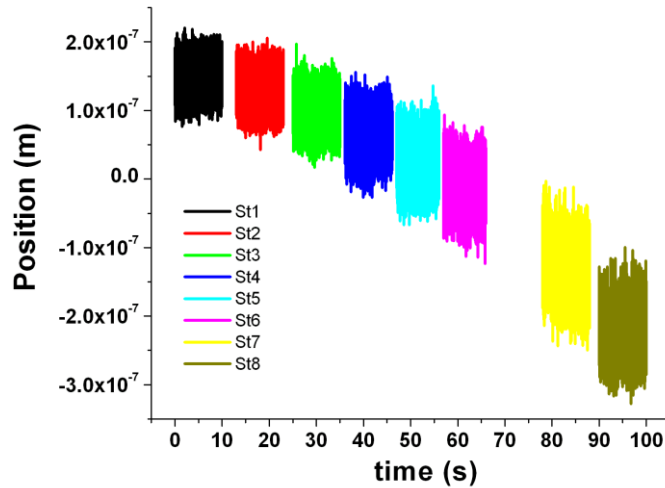


Fig. 3.2. Different stretching trajectories. Each color corresponds to a stage. Position has been calibrated.

Then, for each different stretching a histogram of position is done. This is an important point, because Boltzmann statistics permits to link this histogram with the potential seen by the particle [10]:

$$p(x) \propto \exp[-U(x)/kT] \quad (3.4)$$

where $p(x)$ is the probability of the particle to be in x , $U(x)$ is the potential seen by the particle, k is the Boltzmann constant and T is the temperature of the sample. Different potentials are shown in fig. 3.3.:

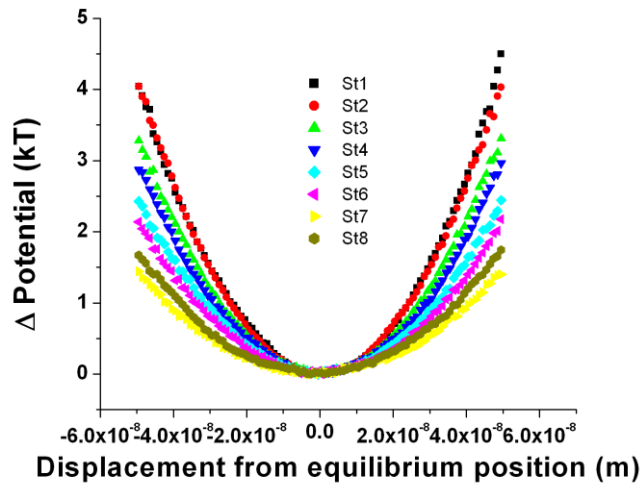


Fig. 3.3. Potentials saw by the bead at different stretching. Note how potential is narrower when stretching is increased.

But this potential is composed of different terms, in first approximation, [11], we can consider DNA as a perfect string, as the optical trap, then it is possible to write the following equation:

$$U(x) \propto \frac{1}{2}\kappa x^2 + \frac{1}{2}\alpha(x - x_0)^2 \Rightarrow F(x) = -\frac{dU(x)}{dx} \propto -\kappa x - \alpha(x - x_0) = -(\kappa + \alpha)x + \alpha x_0 \quad (3.5)$$

where $U(x)$ is the total potential seen by the particle, κ and α are the trap and DNA stiffness, and x_0 is the distance between the equilibrium position of the trap and the equilibrium position of the DNA. Then the recoil force acting on the bead is just the derivative of this potential. The calculus of the histogram gave us the total potential on the particle, so calculus of the recoil force can be made by a numeric derivative, this recoil force for different stretching are shown in fig. 3.4.

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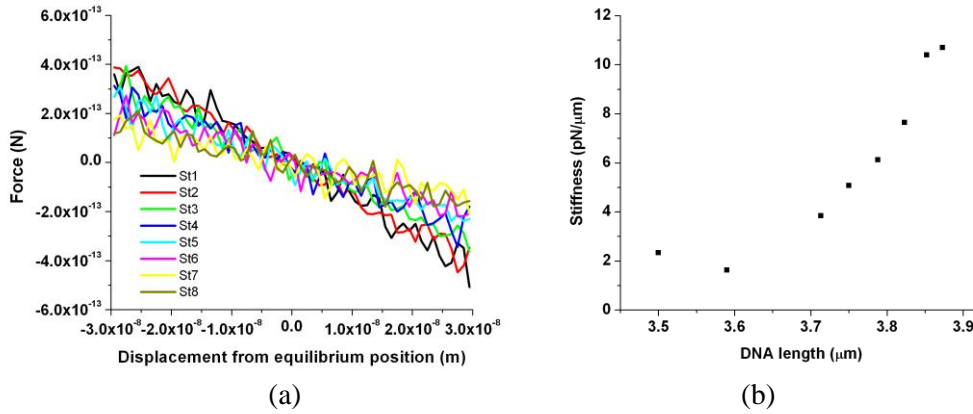


Fig. 3.4. (a) Recoil forces suffer by the bead at different stretchings. Note how slope is increasing with stretching. (b) Stretching curve of DNA. Probe that only one molecule was studied. This curve agrees with WLC and all previous experimental results [12].

Trap stiffness is calculated in the calibration step, therefore DNA stiffness can be known only by a linear fit of the force. Results for different stiffness are presented in table 1.

Table 2. DNA stiffness

	<i>Distance relaxed from St1</i> (nm)	α (pN/ μ m)
St1	0 ± 17	10.70 ± 0.32
St2	90 ± 17	10.40 ± 0.23
St3	213 ± 19	7.65 ± 0.26
St4	250 ± 21	6.13 ± 0.32
St5	288 ± 23	5.07 ± 0.29
St6	323 ± 24	3.84 ± 0.32
St7	352 ± 29	1.63 ± 0.32
St8	373 ± 27	2.34 ± 0.27

These results have to be plotted in order to probe that one and only one molecule has been stretched. This stiffness has been well studied and measured [12] so our results have to agree with them.

3.4. Power Spectrum Density (PSD) for different extensions of the molecule.

PSD of all different stretching are made and plotted in fig. 3.6. (a). The same notation for distance is used.

But it is possible to calculate the corner frequency predicted by WLC, as DNA only acts as a perfect spring, which is a stretching dependent constant, table 3:

Table 3. Corner frequency dependence with stretching.

	f_c (Hz)
St1	150.9 ± 3.2
St2	147.8 ± 2.3
St3	119.9 ± 2.6
St4	104.5 ± 3.2
St5	93.7 ± 2.9
St6	81.3 ± 3.2
St7	58.8 ± 3.2
St8	66.1 ± 2.7

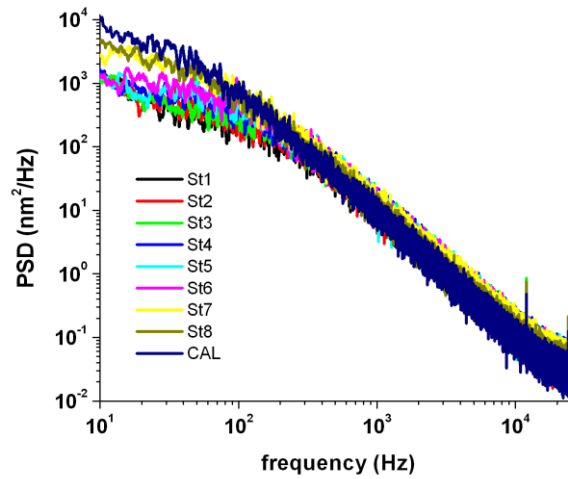


Fig. 3.6. PSD of DNA attached bead and free bead. CAL is the same that fig 3.1. It is shown that St curves do not correspond to WLC prediction. There is no flat zone before corner frequency.

For a better view, St2, St6 and St7 was selected and focused before the corner frequency appears, fig.3.7:

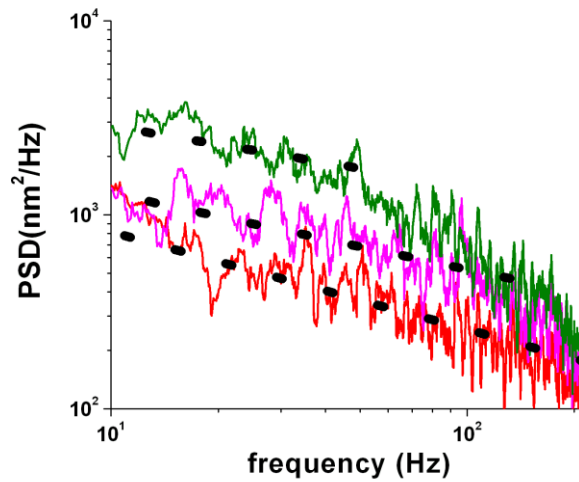


Fig. 3.7. Zoom over low frequencies of three different St curves, St2 (red), St6 (pink) and St7 (green). Black dot make a linear fit in this region. This show how slope is decreasing when DNA is relaxed

4. Discussion

From calibration, although the low power of trapping laser decreases the trap stiffness and then the corner frequency, it was demonstrated that the PSD is well adjusted to a Lorentzian curve fig.3.1. After that, studies in the histogram give us the different stiffness at different stretching, checking only one single molecule was in the experiment and probing that WLC model is a good approximation when stiffness is the key. Otherwise, it gave the different corner frequency which say where the flat zone of the Lorentzian must to start. But the same results which agree with the WLC in stiffness don't agree when PSD is plotted. Theoretically a new Lorentzian with different corner frequency (depending on stretching) has to appear, but between experimental resolution (10Hz) until corner frequency appears a non flat straight line whose slope increases with stretching.

Mathematically, WLC only adds an extra stiffness to the trap stiffness which doing a parallelism with eq. 3.1 gives the following one:

$$\gamma \dot{x}(t) + \kappa x(t) + \alpha [x(t) - x_0] = \sqrt{2kT\gamma\eta(t)} \quad (4.1)$$

$$PSD(f) = |\tilde{x}(f)|^2 = \frac{kT}{2\pi^2\gamma(f^2 + f_c^2)} \quad (4.2)$$

where now $f_c = (\kappa + \alpha)/2\pi\gamma$, same notation than before was used. Here the harmonic trap potential approximation, Stokes regime in bead and neglecting viscosity effects of DNA and inertial terms are used.

Then no explanation for this new effect is inside the WLC model, new term has to be included:

$$\gamma\dot{x}(t) + \kappa x(t) + \alpha(x(t) - x_0) = \sqrt{2kT\gamma}\eta(t) + \chi(t) \quad (4.3)$$

$$PSD(f) = |\tilde{x}(f)|^2 = \frac{|\sqrt{2kT\gamma}\eta(f) + \chi(f)|^2}{4\pi^2\gamma^2(f^2 + f_c^2)} \quad (4.4)$$

where $\chi(t)$ is a new stochastic function DNA dependent. This new function maybe can explain how PSD of stretching DNA is changing with force.

5. Conclusions

In this work DNA stretching measurement was developed by optical trapping technique. Previous studies have considered DNA as a polymer, applying WLC model to understand its physics. This model works for DNA stiffness but we have probed that it does not work for understanding the PSD at different stretching stage. This is really important, because during all processes DNA has to lose its tertiary structure and these effects can appear. At the end PSD is how the energy of a signal is distributed in frequencies, so it is not the same to have a completely white spectra that have a “special range of frequency” which carry more energy. This range can be related in some form to the enzymes which work with DNA improving its efficiency. Future work will concentrate on removal of PSD data of free bead in the trap form PSD data of the bead attached with DNA, and then PSD of previously defined stochastic function $\chi(t)$ will be achieved.

Acknowledgments

This research was funded by the Spanish Ministry of Science and Innovation (Grant No. MICINN FIS2008-00114) and Fundació Cellex Barcelona. I am indebted to my supervisor Prof. Dmitri Petrov for his help and encouragement throughout the course of this work. I also want to thank Filip Beunis, Satish Rao, Prof. M. Rubí (UB) and the rest of the members of my group at ICFO for their helpful conversations and comments.

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