Biopolymer Elasticity

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Abstract

In recent years molecular elasticity has emerged as an active area of research: there are experiments that probe mechanical properties of single biomolecules such as DNA and Actin, with a view to understanding the role of elasticity of these polymers in biological processes such as transcription and protein-induced DNA bending. Single molecule elasticity has thus emerged as an area where there is a rich cross-fertilisation of ideas between biologists, chemists and theoretical physicists. In this article we present a perspective on this field of research.

Polymers of different flexibilities are vital to life. Some polymers are as stiff as needles, others are as flexible as thread and still others are like twine, neither too stiff nor too floppy. The cytoskeletal structure of the cell, which gives rigidity to the cell, consists of semiflexible polymers – like Actin, Microtubules and intermediate filaments. The polymer which carries the genetic code, the DNA (Deoxyribo Nucleic Acid), is a semiflexible polymer (*See* Box 1). A typical human DNA in a cell is about a meter long and it is rather amazing that such a long molecule is packed into a cell nucleus of the size of a few microns. This efficient packaging is a mystery and raises questions about the elasticity of DNA. DNA also assumes various different forms depending on the function it needs to perform – for instance, it is unwound during replication so that the genetic code can be accessed and copied. At other times the DNA assumes a supercoiled configuration which protects and preserves the genetic code intact till it is needed. Such conformationally distinct configurations are also dictated by its elasticity.

Single Molecule Experiments:

Experimental studies of biopolymer molecules such as DNA have traditionally been limited to samples containing large numbers of molecules. This made it hard to probe the elastic properties of individual biopolymers which are of vital importance to biological processes such as protein-induced DNA bending. It is only quite recently, due to advances in technology that single molecule studies became feasible. It is now possible to design experiments in which single molecules are pulled, stretched and twisted to measure elastic properties. In a typical single molecule experiment, one considers a polymer molecule suspended between a fixed surface and a force sensor of some kind (*See* Fig. 1). The force sensor is a bead in

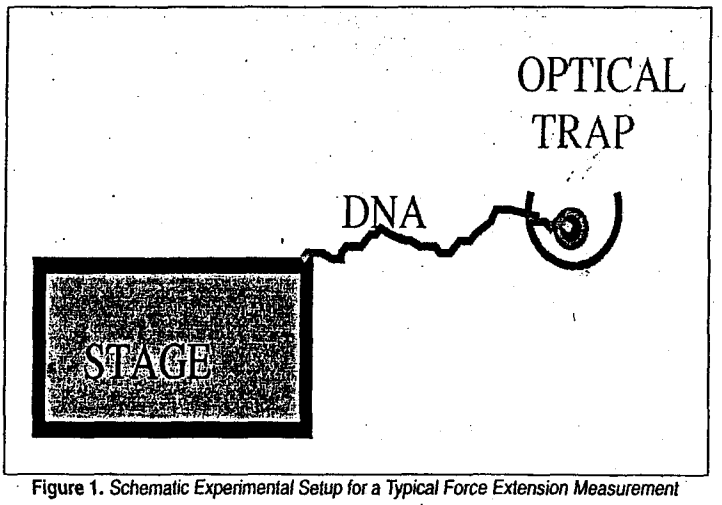

Figure 1. Schematic Experimental Setup for a Typical Force Extension Measurement

a laser trap or a flexible cantilever of an atomic force microscope and the molecule under consideration can range from a DNA in a simple random coil configuration to a globular protein in a unique three-dimensionally folded form. For instance, one can study the "equation of state" of a semiflexible polymer by measuring its extension as a function of applied force. One can also tag the ends with fluorescent dye and determine the distribution of end-to-end distances. We learn a lot from such experimental studies about biologically relevant mechanical properties of these polymers.

Such single molecule micromanipulation studies have opened up a vast area of cross-fertilisation of ideas between biologists, chemists and theoretical physicists. Results of single molecule experiments have posed challenges to theoretical physicists who have combined ideas from statistical mechanics, quantum mechanics, differential geometry and topology to get a proper understanding of the field (*See Box 2*).

Force-Extension Curves: Experiment And Theory

Carlos Bustamante and his colleagues directly measured the elasticity of a single DNA molecule by attaching one end of the molecule to a glass slide and the other end to a micron size magnetic bead. A stretching force was applied to the DNA molecule by applying a magnetic field gradient and the corresponding extension was measured by observing the position of the bead under an optical microscope. The mechanical response of such a system was thus probed in the form of a force-extension curve.

The experimental curves were compared first against the simplest theory available for a polymer-the freely jointed chain (FJC) in which the polymer is viewed as a chain of rigid rods connected by revolving pivots. While the experimental curves agreed well with the FJC model at low forces, the comparison showed considerable discrepancies between theory and experimental curves in the intermediate and large force regime. This pointed towards a deficiency in the theory which was subsequently remedied by Marko and Siggia who used the Worm Like Chain (WLC) model. In the WLC model one takes into consideration the bending energy ($\varepsilon = \frac{1}{2} A \int_0^L ds\kappa^2$ with $A = L_p k_B T$ the bending modulus) proportional to the square of the curvature κ of the space curve representing the polymer of contour length L kept at a temperature T. The WLC model provided excellent quantitative agreement with the experimental force-extension curves (*See Fig. 2*). This pointed to the fact that a DNA molecule needs to be viewed as a semiflexible (partly flexible and partly stiff) polymer rather than a completely flexible one.

Twisting Single Molecules:

In the experiments of Strick and collaborators the ends of a single molecule of double stranded DNA are attached to a glass plate and a magnetic bead. Magnetic fields are used to control the orientation of the bead and magnetic field gradients to apply forces on the bead. By such techniques the molecule is stretched and twisted and the extension of the molecule is monitored by the location of the bead. A typical experimental run measures the extension of the molecule as a result of the applied twist and force. These experiments are important to understanding the role of twist elasticity in biological processes like DNA replication.

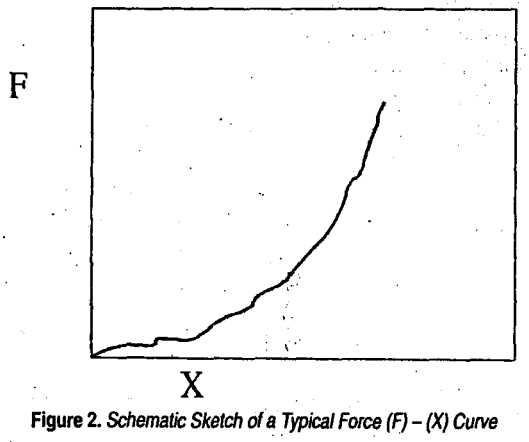

Figure 2. *Schematic Sketch of a Typical Force (F) – (X) Curve*

In laboratory and biological conditions the thermal fluctuations of the DNA molecule are important and it is necessary to take these into account to understand the experiments of Strick *et al*. To determine the response of a DNA molecule to twist and stretch one has to calculate its partition function: sum over all possible configurations of the molecule with Boltzmann weight exp[—*E/kT*]. As Strick *et al* remark that is a tall order, not likely to be filled anytime soon. Computer simulations of the system are possible and have been done. These simulations, which incorporate realistic features like self-avoidance, agree well with laboratory experiments and can be viewed in two ways. From the point of view of a laboratory experimenter they are like theoretical models. From a theorist's standpoint simulations can be viewed as controlled experiments. The main theoretical difficulty is the incorporation of self-avoidance, which is present in a real DNA molecule: the conformation of the molecule must never intersect itself. Such a constraint is virtually impossible to handle theoretically, and one is forced to resort to simpler models. Nevertheless, researchers have attempted to model such a system by incorporating a twist term in the energy functional

Tech Edge

How brain cells chatter:

Paris: French scientist using an innovative microscopic scanning says they have discovered that nerve cells almost buzz with molecular agitation when they communicate with each other.

The work sheds light on how cells operate at the synapse – the minute gap between neurons, as nerve cells are called.

Neurons communicate by sending chemical signals across the synapse, which then latch on specific targets, known as receptors, on the membrane of the adjoining cell.

The chemicals activate an electrical signal in that cell, which then sends on a chemical signal to its neighbour, and so on down the line, eventually triggering the desired response or movement in the finger, hand, limb or other organ.

Until now, little was known about receptor movement, and it was thought that these vital "locks" that open to the heart of the cell were largely static.

But nanotechnology, harnessed to a video camera by French researchers, shows the receptors to be extraordinarily active and that they may even move around dynamically on the membrane surface.

The discovery is important, because it highlights the complex, highly mobile mechanism in which a receiving cell is able to detect just a single molecule.

The team, led by Antoine Triller, head of an inserm unit that specialises in synapse research, and Maxime Dahan. of Kactler Brossel Labratory at Paris's Ecole Normale Superieure, publishes its work in Friday's issue of Science, the US scientific weekly.

Their observations were made on spinal cord tissue from rats, and used a probe called quantum dots – fluorescent semiconductors, with a cadmium-selenium core and a zinc sulphide shell – to tag receptors for glycine, a key synapse signaling chemical.

The "dots" measure just 5 to 10 billionths of a meter across, and are just a quarter of the smallest nanoparticle tracers used so for. Those particles, made of gold or latex, range from 40 to 500 billionths of a meter, which means they are too big to revel the single molecule properties of living cells

– Courtesy: AFP

and trying to explain the results of the experiments. Although the agreement with the experimental curves is good there are some issues related to self-avoidance of the polymer which are still poorly understood and one needs to introduce an adjustable cutoff parameter to fit the experimental curves. The significance of this cutoff parameter is not entirely clear and there are open issues yet to be understood.

Finite Size Effects in Single Molecule Experiments

In the past, experiments on biopolymers were confined to studying their bulk properties, which involved probing large numbers of molecules. The results of these experiments could be analysed by using the traditional tools of thermodynamics. However, when one micromanipulates single polymer molecules the usual rules of thermodynamics applicable to bulk systems no longer hold. Single molecule experiments thus provide physicists with a concrete testing ground for understanding some of the fundamental ideas of statistical mechanics. In particular, fluctuations about the mean value of a variable play an important role due to the finite extent of the molecule. For instance, it turns out, that an experiment in which the distance between the ends of a polymer molecule is fixed (an isometric setup) and the tension fluctuates yields a different "equation of state" from one in which the tension between the ends is held fixed (an isotensional setup) and the end-to-end distance fluctuates. This asymmetry can be traced to large fluctuations about the mean value of the force or the extension, depending on the experimental setup. These fluctuations vanish only in the thermodynamic limit of very long polymers. A similar effect is seen in experiments involving twisting DNA molecules. If the DNA is short enough, experiments with a fixed torque and a fluctuating twist give a different Torque-Twist Relation from those with a fixed twist and a fluctuating torque.

Equilibrium and Nonequilibrium Statistical Mechanics: A Connection

We will now come to an interesting connection between equilibrium and nonequilibrium statistical mechanics which has emerged in the past few years and its relevance to single molecule experiments.

In recent years Jarzinsky has proved a remarkable equality relating work done in a series of finite time, *nonequilibrium* measurements and the *equilibrium* free energy difference between two given configurations of a system. What is most remarkable is, unlike linear response and the fluctuation-dissipation theorem (*See* Box 3) which relate equilibrium statistical mechanics to *nonequilibrium* processes close to *equilibrium*, this relation is valid arbitrarily far from *equilibrium*.

Let us consider a finite classical system that depends on an external parameter λ. Let the system come to equilibrium with a reservoir at a temperature T. If we now switch the external parameter from an initial value $\lambda = 0$ to a final value $\lambda = 1$ *infinitely* slowly then the work W_∞ done on the system is given by:

$$W_\infty = \Delta F = F_1 - F_0$$

where F_λ is the Free energy of the system at a temperature T at a fixed value λ. Let us now consider switching the parameter A at a finite rate. In such a situation the system is no longer in quasistatic equilibrium with the reservoir at every stage. The work done in such a case *depends* on the microscopic initial conditions of the system and the reservoir. One now needs to consider an *ensemble* of such switching measurements and the total work done is no longer equal to the free energy difference but always exceeds it.

It turns out, however, that a remarkable equality still holds connecting the work done in such a process and the difference between the initial and final values of the free energy. The equality, known as

Jarzynski's equality is given by: $\overline{exp(-\beta W)} = exp(-\beta \Delta F)$. The overbar on the left pertains to an ensemble average over all possible finite time measurement realisations.

Recently Liphardt and collaborators have tested the validity of this remarkable equality via single molecule experimental measurements. They tested the equality by comparing work done in reversible and irreversible unfolding of a single RNA (Ribo Nucleic Acid) molecule.

Small beads were attached at the ends of a single RNA molecule. The bead at one end was held in an optical trap. By measuring the displacement 5 of the bead in the trap and the stiffness constant k of the trap, the force $F = k\delta$ on the molecule was measured. The bead at the other end was held by a micropipette and attached to a piezoelectric device which enabled the position of the bead to be controlled by varying the voltage. The position z of the second bead relative to a given reference point was used to measure the extent of unfolding of the molecule. The work W done was measured by integrating the product of the force and the displacement and plotted as a function of the extension z.

The experiment was repeated at varying rates of pull. There were essentially two classes of

Tech Edge

In grid computing, US lagging behind Europe

When Swiss-based pharmaceutical giant Novartis needed a new supercomputer for designing drugs, it found it already had one. It was hidden in the unused computing power the firm had available in the thousands of PC's already being used in its offices. Novartis used American Software Technology to harness the power of its office PCs, but European and American scientists and government officials say Europe is moving faster than the US to capitalise on the approach, known as grid computing. Grids lash individual together, toping unused power to tackle complex computing chores beyond the scope of isolated processors. They are begin used by scientist and corporations for many applications, including building low-cost supercomputers and creating work groups that can span cities or even the globe. "Europe has decided that this is a competitive advantage", said Peter A Freeman, assistant director of the National Institute Foundation (NSF). "And they are going after it". Europe's rush to grids underscores cultural and political differences between it and the US. While American universities and firms often lead the innovation parade, the US sometimes becomes hamstrung in putting new technologies into use. In contrast, European governments are more effective in deploying unified standards and concentrating on technologies that appear to offer an economic advantage. With grid computing, Europe may have an 18-month lead in deploying the advances in practical ways. While the US is beginning to respond to a report in February from the NSF advisory panel on cyber infrastructure urging co-ordinated investment in grid technologies, the EU is preparing to start two major initiatives in early 2004. One, called Enabling Grids for E-science in Europe, aims to build the largest international grid infrastructure, operating in more than 70 institutions, providing 24-hour grid service and a computing capacity comparable to 20,000 powerful PCs. The other is a distributed supercomputing project, led by France's Research, that will connect seven super computers at optical network speeds. The US is ahead on one front. It has made the most progress in deployment of computing grids for scientific applications like studying earthquake risks. Next Year, the Tera Grid project is expected to offer computing speeds of up to 20 trillion mathematical operations a second and the ability to store a petabyte of information. Europe's equivalent effort, Openlab, involving IBM and a research center in Switzerland for the Geneva based European Organization for Nuclear Research, known by its French acronym, CERN, is not expected to reach the same level before 2005. But the Europeans are racing ahead in developing faster optical networks. A CERN- Cal tech team set an Internet 2 Land Speed Record recently by transferring 1.1 trillion bytes of data in less than 30 minutes.

– Courtesy: NYT News Service

measurements: quasistatic, near equilibrium measurements where the work done was reversible and given by the free energy difference between the two end values of the control parameter. The other class consisted of measurements carried out at faster rates of pull (truly *nonequilibrium* situation) where the molecule does not have time to equilibriate during pulling. The experimenters took an average over all possible realisations of the nonequilibrium measurements and evaluated the quantity $\overline{exp(-\beta W)}$ and compared it against its equilibrium counterpart $exp(-\beta \Delta F)$ and found good agreement within experimental error corroborating Jarzynski's equality.

Concluding Remarks:

In this article we have tried to give a perspective on the growing and exciting area of biopolymer elasticity where there has been a merging of ideas between biologists, chemists and theoretical physicists. On one hand, elasticity experiments on biopolymers triggered by a need to understand its role in complex biological processes such as transcription and gene regulation have inspired physicists to come up with models that capture the essence of the problem and at the same time give quantitative explanations for force-extension and twist-extension measurements. On the other hand, predictions made by theoretical physicists have suggested new experiments. Computer simulations have played a two fold role: they have provided researchers with results of "controlled experiments" which are ideal for theoretical model building. At the same time, the results of the simulations when tested against real experiments enable experimenters to identify the key factors which control the experimental results. Apart from biological relevance, biopolymer elasticity experiments have provided a testing ground for theoretical physicists for deeper issues related to connection between equilibrium and nonequilibrium statistical mechanics.

BOX I: Mean Square End-To-End Separation Of A Randomly Lying Cord

Typical chromosomal DNA molecules are long (contour length $L \approx 50$mm) and when viewed under a microscope look like random coils and the statistics of their ensemble of equilibrium configurations is like that of a random walk. The mean square distance between the ends of a molecule grows in proportion to its contour length L:

$$<R^2> = 2L_pL.$$

The constant L_p is the persistence length which is the length over which the DNA molecule can be considered to be approximately straight.

In general, for a seminexible polymer, the mean square separation between its ends predicted by the most popular theoretical model of biopolymer elasticity, the Worm Like Chain model is:

$$< R^2 >= 2L_p^2[L/L_p + e^{-L/L_p} - 1]$$

which in the limit of $L/L_p >> 1$ as for a DNA, goes as:

$$< R^2 >= 2L_pL.$$

In the other limit, that is, for a stiff biopolymer like an Actin filament the mean square separation between ends grows ballistically with the contour length

$$< R^2 > \sim L^2$$

There is an experiment that one can do using a fairly low-tech setup to check the statistics of end-to-end separation of polymers. This 'scaled up' experiment tells us that the statistics of separation between the ends of a cord is the same as one gets for a seminexible polymer. The original experiment due to Lemons and Lipscombe (*See* Suggested Reading List) consisted of taking some short ($\approx 0.1m$)

and some long ($\approx 0.8m$) segments of a bungee cord of length ($=0.8m$) and persistence length ($\approx 0.25m$). To mimic the effect of randomisation, the cord was dropped onto the floor from a height of 2m. Positions were marked off on the cord corresponding to different segment lengths L. After dropping the cord, the distance R of separation between the $L = 0$ end and one of these marks was measured and for each such segment length the procedure was repeated about 40 times. $< R^2 >$ was then plotted against L and the results agreed well with the theoretical expression

$$< R^2 >= 2L_p^2[L/L_p + e^{-L/L_p} - 1],$$

The persistence length or the rigidity parameter L_p was determined from the fit of the data against the theoretically predicted form.

BOX 2: Link=Twist+Writhe

DNA can form a closed loop. Since it is a double stranded helix, the linking number Lk or the number of times one strand wraps around the other is fixed. In 1971 Fuller noticed an interesting connection: the linking number Lk of a closed ribbon can be decomposed into the "Writhe" Wr of its backbone plus a locally determined "Twist" Tit; :

$$Lk = Tw + Wr.$$

Link Lk counts the number of times that one strand winds around the other. It is an integer and clearly a topological quantity and does not change unless you cut and paste the strands. Writhe Wr is a nonlocal quantity which measures the wandering of the tangent vector to the polymer backbone and it depends only on the central curve or the backbone of the ribbon representing the polymer. Twist Tw is a local quantity that measures the integrated angular velocity of a strand about the backbone of the polymer. Both Tw and Wr can change so as to keep Lk unchanged. Fuller noticed this relation in the context of a question related to supercoiled double stranded DNA rings raised by J. Vinograd, a molecular biologist. This is an example where molecular biology has stimulated a mathematical line of enquiry.

BOX 3: Fluctuation-Dissipation Theorem

When you turn on a radio you can sometimes hear a noise which can be traced to the irregular motion of electrons. Nyquist was the first to recognise the connection between such thermal noise and the impedance of a resistor across which an irregular voltage difference is induced due to thermal motion of the electrons. One sees a similar connection in the context of Brownian motion (*See* S. Ramaswamy's article mentioned in the Suggested Reading List). The generalised theorem relating the fluctuations of a physical variable of a system in equilibrium to dissipative processes in the system subjected to an external force driving it slightly away from equilibrium, is called, the Fluctuation-Dissipation Theorem. The Fluctuation-Dissipation Theorem works in the linear response regime in which the response of a system to an external stimulus is directly proportional to the strength of the stimulus.

Suggested Reading

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