Chemistry C3102-2006: Polymers Section

Dr. Edie Sevick, Research School of Chemistry, ANU

2.0 Thermodynamics of an Ideal Chain: Stretching and Squashing

In this section, we begin to investigate the thermodynamics of polymers, starting first, with an isolated ideal chain. We begin with the Helmholtz free energy, F, which corresponds to the available work at a constant temperature:

$$\Delta F = \Delta U - T\Delta S, \tag{1}$$

where U is the internal energy of the chain, T is the temperature which is held constant and S is the entropy. Examples of energies which contribute to U range from Lennard-Jones interactions to complex interactions which scientists might propose, all of which are of the form $U = f(\mathbf{r}_{ij})$ where $\mathbf{r}_{ij}$ is a vector of distances between monomers, solvent, or other particle or surface which might be included in the system. By definition, an ideal chain suffers NO interactions and contributes nothing to the internal energy at a constant temperature. Consequently, if we take an ideal chain and end-tether it to a phantom wall, or change the solvent, then the change in the internal energy of the system is 0 under isothermal conditions: $\Delta U = 0$ [1]. On the other hand, the chain has considerable disorder, measured by the number of configurations that it can adopt and quantified by the thermodynamic quantity entropy. If we were to hold the two ends of the chain a distance Na apart so that the chain was taut, we would considerably decrease the number of configurations that the chain could adopt. If S_1 is the entropy of the ideal chain in its relaxed, or natural state, and S_2 the entropy of the chain in its taut state, then $S_1 >> S_2$ or $\Delta S = S_2 - S_1$ would be negative, and by the above equation, ΔF, would be positive. That is, in stretching the chain to its taut state, we would be performing work on the system to increase the free energy of the system. If we were to release the ends of the chain, the chain would spontaneously relax to its natural state that maximises the number of available configurations; that is the system maximises its entropy and minimises its free energy. Thus, in order to describe the thermodynamics of an ideal chain, we need only quantify the entropy of the chain. In this section, we will investigate the thermodynamics of an ideal chain when it is stretched and when it is squashed. The force of stretching and squashing is determined solely by entropy- such forces are referred to as entropic or elastic forces.

Boltzmann's Principle (after Ludwig Boltzmann (1844-1906)) states that the entropy of the system is $S = k_B \ln[\Omega]$, where Ω is the number of configurations of the system and k_B is Boltzmann's constant:

$$\begin{aligned} k_B &\equiv 1.381 \times 10^{-23} J/K \\ &\equiv \frac{R}{N_a} = \frac{8.314\text{J mol}^{-1}\text{K}^{-1}}{6.022 \times 10^{23}\text{mol}^{-1}}. \end{aligned}$$

Imagine that we could actually count the number of configurations of a chain in its natural and fully-stretched states, then the change in entropy would be $\Delta S = k_B \ln[\Gamma/\Gamma_0]$ where Γ is

[1] Like an ideal gas, if there is a temperature change, $\Delta U = C_v \Delta T$

the number of taut configurations and Γ_0 is the larger number of configurations in the chain's natural state.

EXAMPLE PROBLEM

•• What is the change of entropy associated with an ideal chain of N monomers forming a ring?

From Boltzmann's principle, we find that the entropy change for ring formation is

$$\Delta S = S_{ring} - S_0 = k_B \ln (\frac{\Gamma_{ring}}{\Gamma_0}) \tag{2}$$

where the subscript 0 indicates an FJC chain possessing the same number of monomers, of the same size, but whose ends are not constrained. Γ_{ring} and Γ_0 are the total number of configurations of the ring and the unconstrained chain, respectively. Following section 1.2, we can express Γ in terms of the number of possible random walks that arrive at a destination m. In 1-dimension, the total number of configurations available to the random walker is 2^N, as at each step the walker has 2 possible directions. In order for the walker to form a ring, he must return to his starting point, or in the language of section 1.2, $m = 0$ and $n^+ = n^-$. The number of such ring configurations is

$$\frac{N!}{(\frac{N}{2})!(\frac{N}{2})!} \tag{3}$$

Therefore the probability of observing a random walker returning to his starting point (in 1-dimension) is

$$p_{ring}^{1-D} = \frac{1}{2^N} \times \frac{N!}{(\frac{N}{2})!(\frac{N}{2})!} = (\frac{2}{\pi N})^{1/2} \tag{4}$$

where the last term on the right hand side resulted from application of Stirling's approximation, which is valid in the limit of large N. You should see that this formula also results from eq. 29 in the notes, by replacing m with 0. Likewise, you can transform from discrete destinations to a continuous, end-to-end distance in three dimensions as achieved in Section 1.2. The result is that the probability of observing a ring in three dimensions is

$$p_{ring}^{3-D} = (\frac{3}{2\pi N a^2})^{3/2}. \tag{5}$$

Therefore, the entropy change upon ring formation of a chain of N statistical monomers, each of size a, is

$$\begin{aligned} \Delta S &= k_B \ln p_{ring}^{3-D} = \frac{3k_B}{2} \ln (\frac{3}{2\pi N a^2}) \\ &= \frac{3k_B}{2}[\ln (3) - \ln (2\pi N a^2)]. \end{aligned}$$

As N is large, $\ln (3) << \ln (2\pi N a^2)$, and ΔS is negative, that is, entropy is lost in the process of ring formation, and as $\Delta F = -T\Delta S$, the free energy of the ideal chain increases as it is confined to a ring conformation.

2.1 Stretching a chain

Take an ideal chain, grab each of the free ends, and then isothermally extend or stretch the chain by pulling the ends to progressively larger values of R, the end-to-end distance. We want to know the force needed to stretch the chain to half of its contour length. First, lets express the above equation in differential terms: $dF = -TdS$, where $dU = 0$ as there is no change in temperature. Then, since work, w, is equal to the force, f, integrated over the distance, $\int_0^R dRf$, we can write

$$f = -\frac{dF}{dR} = T\frac{dS}{dR} \tag{6}$$

We already have an expression of the entropy in terms of the end-to-end distance from the Gaussian distribution of end-to-end distance, $p(R)$:

$$S(R) = k_B \ln\left(p(R)\right) = k_B \ln\Big[(\frac{3}{2\pi Na^2})^{3/2} \exp\left(-\frac{3\mathbf{R}\cdot\mathbf{R}}{2Na^2}\right)\Big], \tag{7}$$

where I write $R^2 = \mathbf{R}\cdot\mathbf{R}$, so thht you can see where the vector quantity arises after we take a derivative. We can simplify this to

$$S = k_B \ln(\frac{3}{2\pi Na^2})^{3/2} - k_B(\frac{3\mathbf{R}\cdot\mathbf{R}}{2Na^2}) \tag{8}$$

Taking the derivative of S with respect to $\mathbf{R}$ gives $-3k_B\mathbf{R}/(Na^2)$ as the first term on the RHS vanishes as it has no R dependence. Thus, the entropic or elastic force needed to stretch a chain to an end-to-end distance $\mathbf{R}$ is

$$\mathbf{f} \sim -3k_BT\frac{\mathbf{R}}{Na^2} \tag{9}$$

Notice that $\mathbf{f}$ is a vector quantity, and in the direction opposite to the end-to-end vector. In other words, $\mathbf{f}$ is a restoring force.

EXAMPLE PROBLEMS

• **An ideal polymer chain is end-tethered to a fixed wall and a weight of mass m is suspended from the free-end of the chain, as pictured below. The weight is sufficiently small that the elongation of the chain, R, is between the natural size of the chain and its contour length, $\sqrt{(N)}a < R < Na$. Derive an expression for the chain elongation, x, as a function of the mass of the weight, m, under isothermal conditions**

First, we assume that the force of extension or stretching force is entirely entropic. We can derive the force, f, versus extension, R, relation starting from simple thermodynamics:

$$F = U - TS,$$

where the elastic force, f, is

$$f = -\frac{dF}{dR} = T\frac{dS}{dR}$$

The entropy is given by Boltzmanns principle and the Gaussian probability distribution

$$S(R) = k_B \ln\left(P(R)\right)$$

$$\frac{dS}{dR} = k_B\frac{d}{dR}\ln\Big[\Big(\frac{3}{2\pi Na^2}\Big)^{3/2}\exp\Big(-\frac{3R^2}{2Na^2}\Big)\Big].$$

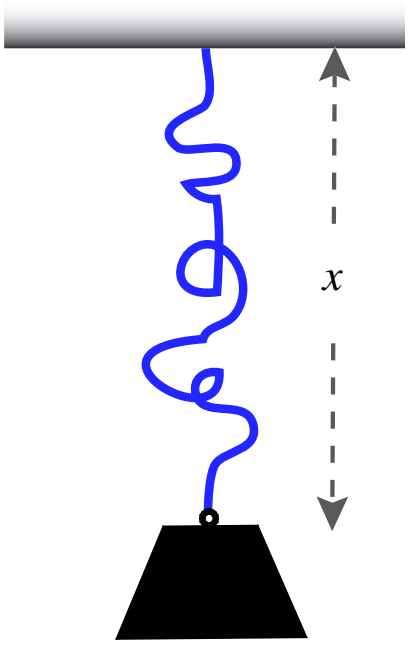


Figure 1: Schematic of an end-tethered ideal chain, extended a distance x under the action of a weight, attached to its free end.

Using the identity $\ln(ab) = \ln a + \ln b$, the last equation becomes

$$\frac{dS}{dR} = k_B \frac{d}{dR}\left[\left(\frac{3}{2\pi N a^2}\right)^{3/2} - \frac{3R^2}{2Na^2}\right],$$

or

$$\frac{dS}{dR} = -k_B \frac{3R}{Na^2}.$$

Therefore, the equation for the force versus extension is

$$f = -k_B T \frac{3R}{Na^2}.$$

Now the force on the chain is simply the mass of the weight times gravitational acceleration, or $f = mg$, so the sought expression is

$$mg = -k_B T \frac{3R}{Na^2}.$$

• Continuing with the weight attached to the free-end of the chain $\cdots$ what happens to the extension of the chain when the ambient temperature is increased, and why?

From the above equation, you see that the LHS is constant the weight on the free-end of the chain does not change. Thus, if the temperature is increased, then the extension must proportionally decrease. Thus this above result indicates that a stretched chain will contract upon heating. This beahviour was first noticed in strips of natural rubber by Guch in 1805 and nearly fifty years later, was measured by Joule: this "shrinkage" with temperature is known as the Guch-Joule effect. Why does the chain shrink with temperature?

It is primarily an effect due to entropy, even in systems when $U \neq 0$. You know that entropy rather than interactions dominate because (1) when temperature is increased, the importance of interactions decrease as the magnitude of the interaction, ε, becomes smaller compared with thermal energy,*i.e.*, $\varepsilon/(k_B T) < 1$; and (2) from $F = U - TS$, you see that increasing the temperature increases the role of entropy. When temperature is increased, the effect on each statistical monomer is to increase its thermal energy. In other words each statistical monomer becomes more randomised with increased temperature or the entropy of the chain is increased. We know that the conformational entropy of an ideal chain is decreased as its ends are separated apart: or that the ideal chains entropy increases as its end-to-end distance becomes smaller. Thus, with an increase in temperature, there is a randomisation of the statistical monomers, an increase in the chains entropy and a decrease in the end-to-end distance (the chain shortens).

While the Guch-Joule effect explains the change in size of an elastic chain by varying the temperature (*i.e.* the size depends upon the temperature, using the temperature as the control variable), you can also change the temperature of the chain by varying the size, or stretching the chain (*i.e.* the temperature depends upon the size, using the size of the control variable). If you stretch the chain very quickly (more quickly than the rate at which heat is exchanged so that effectively $\dot{q} = 0$, or in other words, the stretching process is adiabatic), then the temperature

of the chain will increase (try this with a rubber band using your lips to detect temperature change to verify this). This is opposite to what happens with an ideal gas when it is expanded adiabatically which we review briefly here: The first law of thermodynamics is

$$\Delta U = \delta q - \delta w.$$

Recall that the internal energy of an ideal gas can only change with temperature, $dU = C_V dT$, $\delta q = 0$ for adiabatic processes, and that the work done (reversibly) by the gas is $p_{ext} dV = p_{int} dV$, so that the first law reduces to

$$C_v dT = 0 - pdV$$

which, upon insertion of the ideal gas law, becomes

$$\begin{aligned} C_v dT &= -\frac{nRT}{V} dV \\ C_V \frac{dT}{T} &= -nR \frac{dV}{V} \\ C_V \ln\left(\frac{T_f}{T_i}\right) &= nR \ln\left(\frac{V_i}{V_f}\right) \end{aligned}$$

Next, recognise that $R/C_V \equiv \gamma$, which is equal to $2/3$ for ideal monoatomic gases

$$\left(\frac{V_i}{V_f}\right)^{\gamma} = \frac{T_f}{T_i}.$$

Thus if $V_i < V_f$, that is, the gas expands, then $T_f < T_i$ or the gas temperature decreases. Conversely, if $V_f < V_i$, that is, the gas is compressed, then $T_i < T_f$ or the gas temperature increases. You can understand this qualitatively from the first law: if you compress the gas, you must do work on the system with external forces and this work is converted into the internal energy of the gas, and hence the temperature rises.

• Homework Problem: Using the adiabatic expansion of an ideal gas as an analogy, construct an expression for the temperature change of an rubber band (modelled as an ideal chain) as it is adiabatically stretched.

2.2 Squashing a chain

Consider an ideal chain sandwiched between two infinite, impenetrable plates which form an slit of thickness H. We want to know the force needed to compress the plates such that the slit distance is reduced from Na to a. This is an entropic force as there are no interactions between the plates and polymer, except excluded-volume interactions that limit the number of configurations that the chain can adopt. We cannot easily use the above method to determine the force as we do not know how $p(R)$ depends upon the slit distance H. However, we can assume a simple model of a chain on a lattice so that we can "count" the number of configurations available to the chain at any slit thickness.

First, assume that the ideal chain must traverse the scaffolding of a lattice. We could pick any lattice, but a square (2-D) lattice, or 4-coordinate lattice, is convenient to draw (as you will see, the choice of lattice does not matter in the derivation). Each statistical monomer must adopt one of four vertices on the lattice. Thus, starting from the first monomer, the chain has 4 possible vertices to choose from, the second monomer has another 4 possible vertices to choose from, etc. Thus, the total number of configurations available to a chain of N statistical monomers on a 4-coordinate lattice is 4^N. We can now generalise the lattice to any regular lattice with z vertices or a "z-coordinate" lattice, where z^N configurations are available. Now imagine that this lattice is of infinite extent in two directions, but has a thickness H that we will vary.

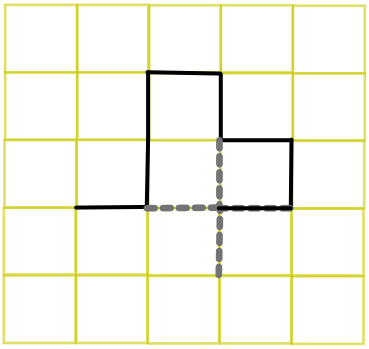


Figure 2: Schematic of counting the configurations of a 2-D chain on a square, or 4-coordinate lattice

How does the thickness of the scaffolding reduce the number of configurations of the chain? As each statistical monomer has z possible vertices to traverse, a statistical monomer which is located at the boundary has only 1 possible vertex - it must "reverse" its direction. Thus, the number of possible configurations is z^{N-X} where X is the number of monomers which contact the boundaries. If ΔS, the entropy change due to confinement, is proportional to the logarithm of the ratio of the number of configurations in an H-thick slit to that in an infinitely-thick slit, then

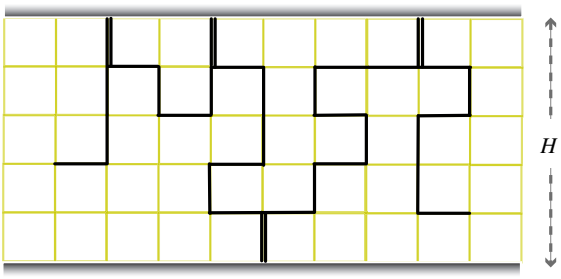


Figure 3: Schematic of counting the configurations of a 2-D chain in a slit formed by inert, but impenetrable walls, The ideal chain must fold-back along itself when it "feels" a wall and loses configurational entropy per wall contact.

$$\Delta S = k_B \ln\left(\frac{z^{N-X}}{z^N}\right) = -k_B X \times \ln(z) \qquad (10)$$

We need to develop an expression that relates X, the number of contacts the chain makes with the boundaries, to H, the separation between the boundaries. If we were to snip the chain at all points of contact with the boundaries, the chain snippets would be on average m

monomers long with end-to-end distances of exactly H. As the chains are ideal, the general scaling relationship for chain size holds: $H^2 = ma^2$. The number of chain snippets is equal to the number of contact with the boundary, or X. The number of chain snippets is N/m, or replacing m with H^2/a^2, $X = Na^2/H^2$. Therefore,

$$\Delta S = -k_B \frac{Na^2}{H^2} \times \ln(z) \tag{11}$$

Now the factor $\ln(z)$ is just a constant, independent of H, so we will drop this factor as we only care about how the force changes with H and not necessarily its magnitude. The change in free energy in going from an infinite slit to one of thickness H under isothermal conditions is $\Delta F = -T\Delta S \sim k_B T \times Na^2/H^2$. Since $F(H = \infty) = 0$, we can equate $F(H) = k_B T \times Na^2/H^2$. Then force of compression at any given H is $f = -dF/dH$, or

$$f \sim k_B T \frac{Na^2}{H^3} \tag{12}$$

EXAMPLE PROBLEMS

•• The free ends of an ideal chain are end-tethered to two parallel, volume-excluding, and thermostatting walls. Construct (graph) the force profile associated with changing the separation of the walls from $H = a$ to $H = Na$.

There are two regimes to consider

- *Chain squashing over $a < H < \sqrt{N}a$* When the open slit is smaller than the natural size of the chain, $\sqrt{N}a$, the number of conformations of the compressed chain is smaller than that of a free, or uncompressed chain. That is, there is a reduction in the entropy of the chain, or an increase in the free energy of the chain, $\Delta F = -T\Delta S$, as $\Delta U = 0$ for an ideal chain. The entropy reduction can be approximated by simply counting the number of times the Gaussian chain contacts one of the plates. Each monomer located at a plate loses a bit of entropy, of order k_B, as demonstrated in the lattice model of section 2.2 of the notes. So we simply need to find the number times a Gaussian chain of N monomers contacts the of the chain and multiply this by k_B to find the decrease in entropy. Any contiguous segment of a Gaussian chain also obeys Gaussian statistics (as long as the segment is sufficiently long). Thus consider that the squashed chain is severed at each of the contact points and let m be the average number of monomers in the segments. Becuase each segment obeys Gaussian statistics, and the end-to-end distance of each segment is determined by the slit separation, H, we can say that $H^2 \sim ma^2$ or that m scales as $(H/a)^2$. The number of times the chain is severed N/m, or $\sim Na^2/H^2$. Thus, the entropy reduction is $k_B T \times Na^2/H^2$. Then,

$$\Delta F = F(H) - F(\infty) \sim k_B T \frac{Na^2}{H^2} - 0 \tag{13}$$

where we set $F(\infty) = 0$ since there is no configurational constraints when $H \to \infty$. The entropic compressing force is then

$$f = -\frac{dF(H)}{dH} \sim k_B T \frac{Na^2}{H^3} \tag{14}$$

where we have dropped all numerical prefactors of order 1. Note that the free energy of the chain increases as the slit distance decreases; *i.e.*, $|dF/dH| < 0$ so that the force is positive, pushing the plates apart. This squashing force holds for H such that $O(100) < m < N$. The lower bound, order of 100, is required so that the segments still obey Gaussian statistics and the upper bound ensures, on average, one contact. This range $O(100) < m < N$ is more informatively cast in terms of H through $m/N \sim H^2/(Na^2)$, or

$$\begin{aligned} O(100) < m < N \\ O(100) < H^2/a^2 < N \\ O(100)a^2. < H^2 < Na^2 \\ O(10)a < H < \sqrt{N}a \end{aligned}$$

For $a < H < 10a$ the finite size of the chain segments plays an important role and the entropic force will not necessarily scale as $1/H^3$. For $10a < H < \sqrt{N}a$, the force scales as $1/H^3$

- *Chain stretching over* $\sqrt{N}a < H < Na$ Entropic or elastic stretching force can be again be approximated using Gaussian statistics. This is done in section 2.1 of the notes, with the resulting force profile being

$$f \sim -k_B T \frac{H}{Na^2}, \tag{15}$$

where again we are not keeping constants of order unity, O(1). Note that the force is negative and in opposition to the squashing force, pulling the plates together.

To plot these force regimes on the same graph, we cast the squashing force, $f \sim k_B T N a^2/H^3$ as

$$\frac{fa}{k_B T} \sim N(\frac{a}{H})^3, \tag{16}$$

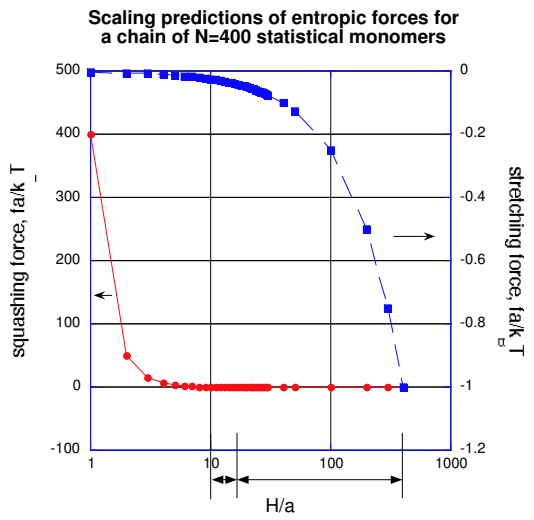
and the stretching force, $f \sim -k_B T H/(Na^2)$ as

$$\frac{fa}{k_B T} \sim -\frac{1}{N}(\frac{H}{a}). \tag{17}$$

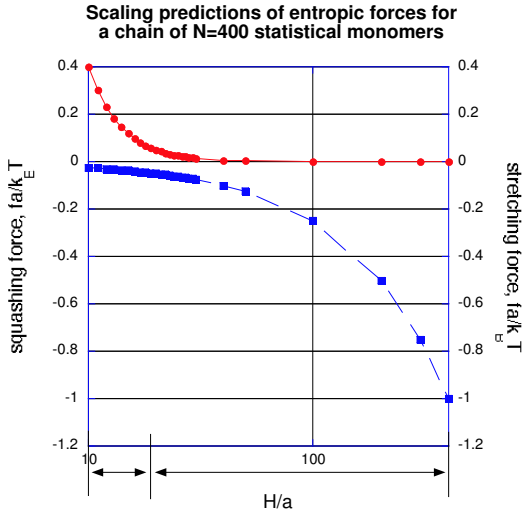
This way you can see that the squashing force is proportional to N while the stretching force is inversely proportional to N. That is, it is harder to squash a bigger chain in the same slit, but easier to stretch a larger chain the same distance.

•• How does the above force profile change if the chain is replaced by a chain of the same exact contour length, but is less flexible or more stiff (has a larger persistence length)? Provide a qualitative description

The force and slit distance were appropriately scaled to dimensionless quantities so that the the plots are unchanged by changing the statistical monomer size, which is twice the persistence length. The slope of the force profile, Fig. 4(d), is proportional to $1/N$ and N, in the stretching and squashing regimes, respectively and independent of the persistence length.



(a)



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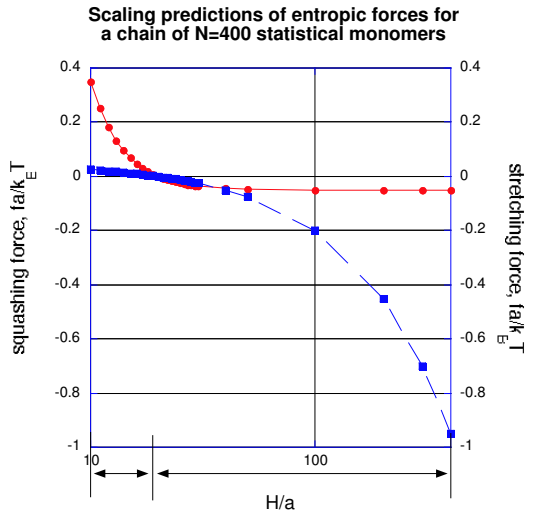

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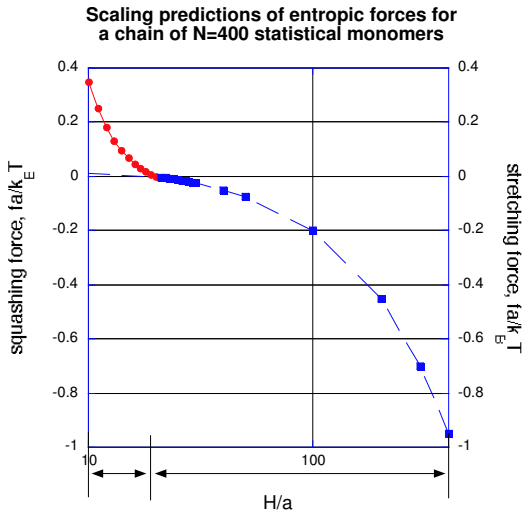


(d)

Figure 4: Versions of the force profile, fa/k_BT versus H/a for squashing (red) and stretching (blue) from (a), (b) draft to final shown in Figure 2(b). In all plots, I adopted $N = 400$ and I assumed that the order of 1 constant in each expression was unity. In (a) I did not worry about the regimes and I plotted both forces over all H/a but noted on the horizontal coordinate that $10 < H/a < \sqrt{N} = 20$ is the squashing regime while $\sqrt{N} = 20 < H/a < N = 400$ is the stretching regime. In subsequent versions (b)-(d), I simply replot versions to get a proper force profile. (b) Here I've changed the horizontal coordinate bounds to $10 < H/a < 400$ so that only the Gaussian squashing and stretching regimes are shown. (c) I added constants to each force profile to make the stretching and squashing forces equivalent (and arbitrarily zero) at $H = \sqrt{N}a$. (d) I eiliminated the stretching points in the squashing regime, and the squashing points in the stretching regime. All done in Kaleidagraph, changing the Kaleidagraph output to .eps format using Illustrator, and inserting them in my Latex document.

•• Answer the above question quantitatively (provide a comparative graph) for a chain whose persistence length differs by a factor of 5

For a more stiff chain, where the persistence length is 5 times longer, the monomer size would also increase by a factor of 5. Therefore at any H/a, the value of fa/k_BT is unchanged, but the force f is reduced by a factor of 5 . This is because a stiff chain has fewer conformations, and consequently less entropy to loose. The range of H over which entropic forces are estimated is increased as the chain is larger in size.

For a more flexible chain, a would decrease by a factor of 5. Again, at any H/a, the value of fa/k_BT is unchanged, but the force f is 5 times as large. This is due to a larger configurational entropy. The range of H over which these entropic forces are estimated is decreased as the chain is small in size.

• • Homework Problem: Construct an expression for the temperature change of an end-tethered chain that is adiabatically squashed between 2 infinite plates.

2.3 Experimental techniques for stretching and squashing single chains

The manipulation of single polymer chains, adsorbed onto a surface has received considerable attention in the past decade, from both theoreticians as well as experimentalists. With the advent of the Atomic Force Microscope (AFM) and Optical Tweezers (OT), scientists are above to impose nanometer scale deformations and measure the resulting forces on the scale of picoNewtons (1 pN= 10^{-12} N). In this section, we will briefly review the techniques of AFM and OT used for micromanilpulation of single polymer chains.

2.3.1 Atomic Force Microscopy

AFM is used routinely for imaging small items, ranging in size from 1 nm to 100 μm, corresponding to say, a single atom, to the width of a human hair. A conventional AFM is based upon a flexible cantilever that is rastered in close proximity over the surface that is to be imaged. Forces, either attractive or repulsive, between the surface and the tip of the cantilever cause the cantilever to deflect. This deflection is measured and converted into an electrical signal. Any chemical heterogeneities in the surface will result in a variation of the force on the cantilever tip as it is rastered over the surface. This variation in force over the rasterised surface is converted into an image.

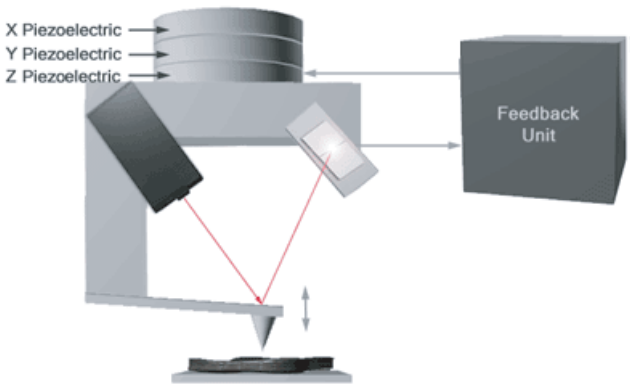


Figure 5: Schematic of an Atomic Force Microscope, from http://www.pacificnano.com/afm-tutorial_afm-instrumentation.html

A schematic of a conventional AFM is given in figure 5. The components of the AFM are:

• **Cantilever** The cantilever is the heart of the AFM . Cantilevers are small, fragile components, with dimensions on the order of $100 \times 20 \times 1\mu$m (length $\times$ width $\times$ thickness). The tip of the cantilevers must be exceptionally small and its size limits the resolution of the image: ideally the tip should be atomically sharp so that it probes the surface with atom-like resolution. In practise, the tip does have appreciable size, and the AFM signal then reflects not the point-like probing of the surface, but rather a convolution of the surface properties over the shape of the tip. (This can cause considerable problem with image interpretation.) Figure 6 are electron micrographs of commercially available cantilevers. The force on the tip of the cantilever causes it to deflect according to Hooke's law,

$$f = -kd$$

, where d is the cantilever deflection and k is a "spring" constant, a property of the cantilever dimensions and material. k varies from cantilever to cantilever and must be calibrated *in-situ*; a typical value of k is 1N/m, or 10^6 pN/μm. There are many cantilevers on the market with different spring constants: you want to choose the cantilever carefully. A cantilever with a large value of k, *i.e.*, a *stiff* cantilever, is used to measure large surface forces.

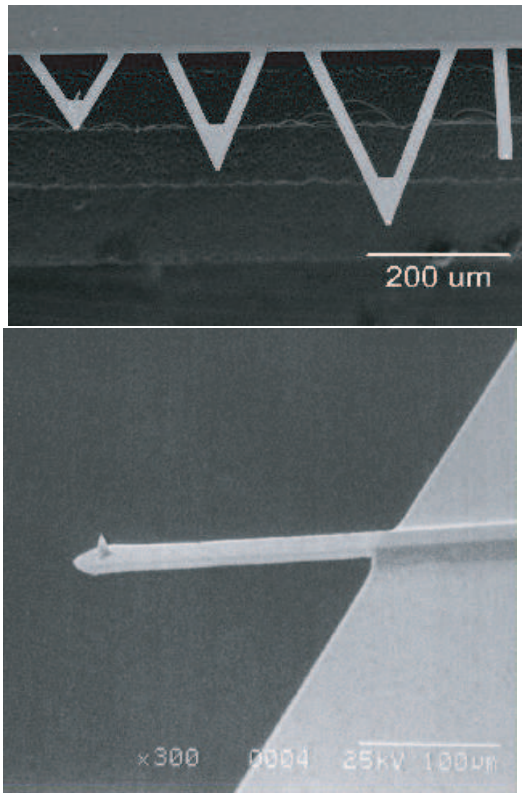


Figure 6: Electron micrographs of AFM cantilevers, from http://www.astbury.leeds.ac.uk

• **Piezoelectric transducers** These electromechanical transducers convert an electrical signal into mechanical motion and are used extensively to precisely translate objects, such as microscope stages, automated pipette/injection delivery systems, *etc.* Piezoelectric crystals are precisely fabricated materials that expand under an applied voltage: they can expand $\mathcal{O}$(1nm) per 1 Volt. Commercially available transducers consist of layers of piezoelectric materials that can be used to translate 1mm per 100 V. These piezoelectric transducers are used to precisely place the the tip of the cantilever a distance z above the surface and to raster or move it in an $x - y$ direction. Usually, the transducers operate on the microscope stage, onto which the substrate is mounted, and the cantilever and deflection sensor are stationary.

• **Deflection sensor** In order to measure the force at any (x, y) point on the surface, we need to be able to measure the cantilever deflection, d. Typically this is done by reflecting a laser beam off of the back of the cantilever onto a photodiode detector surface. As the cantilever deflects, the reflected light beam travels across the surface of detector.

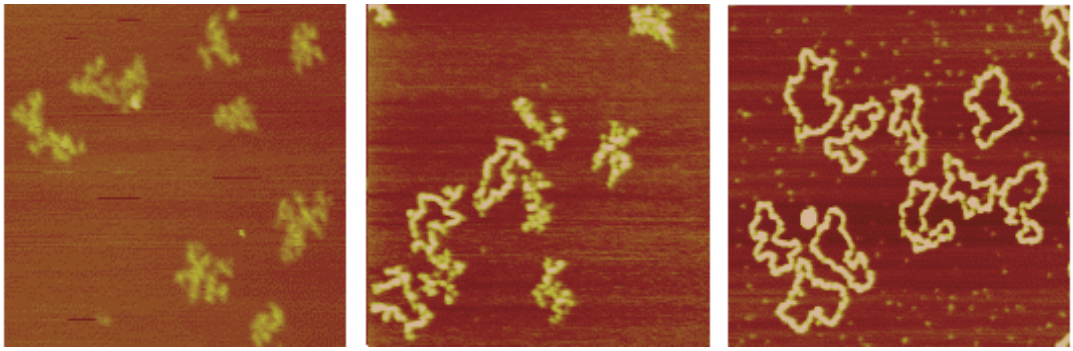


Figure 7: AFM images of circular λ-DNA in single-strand and double-stranded form, by Dr. Genmiao Wang of P&SCM Group of RSC. The AFM image on the right is of *ds*DNA, and the middle image is of the same DNA solution with single-strand-binding (SSB) protein added in less than 1:1:1 bp/SSB stoichiometry, and (far left) SSB added in greater than stoichiometric amount. SSB protein binds to the single-strand form of DNA, stabilising it. Without SSB, the AFM shows opened ringed *ds*DNA, while with SSB protein, the single-stranded form is folded, collapsed upon itself to satisfy complementary pairing of bases. Note that in less than 1:1 bp/SSB amounts, molecules are either fully double-stranded, or fully single-stranded.

AFM is used extensively for imaging surfaces, and figure 7 provides an example of how AFM can be used to image DNA and conformational changes induced by the addition of a specific protein. AFM has also been used in more novel ways.

- Researchers used a cantilever tip to "pick-up" single atoms and deposit the atoms to spell out the name of their company "I B M"; this they did by altering the properties of the cantilever tip, by driving a current through the cantilever. Indeed, one topic of instrumental research is to develop cantilevers than can alter their stiffness *in situ* with the addition of an external field.

- ANU researchers, led by Prof. Richard Pashley at the Department of Chemistry, attached a colloidal particle to the cantilever tip and measured the force between colloid and surface.

- Dr. Tim Senden of the Department of Applied Mathematics, used the cantilever tip to go "fly-fishing" for polymer chains that were adsorbed onto the substrate. The cantilever tip was made of the same adsorbing material as the substrate, so that when the AFM tip was brought in contact with the substrate, there was a good chance that some part of a chain could be adsorbed to the tip. This was arguably the first time that the stretching force of a polymer was measured; however, the disadvantage to this work was that you could not determine the length of contour that was stretched between substrate surface and cantilever tip.

- Nowadays, the big-buzz in single chain stretching is the stretching of biomolecules. Biochemists can functionalise the ends of DNA and attach these reactive ends to substrates and colloidal beads. This same chemistry can be used to tether the ends of DNA to a substrate and an AFM tip. An example of AFM stretching of biopolymers that will probably become a "classic" in this area is described in Rief, M., Gautel, M., Oesterhelt, F., Fernandez, J.M., Gaub, H.E., "Reversible Unfolding of Individaul Titin Immunoglobulin

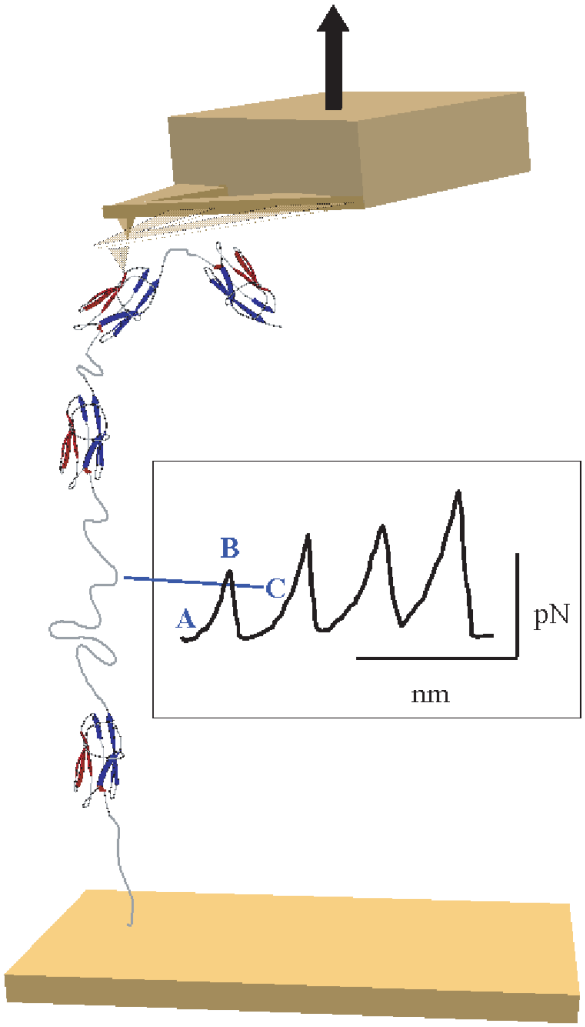
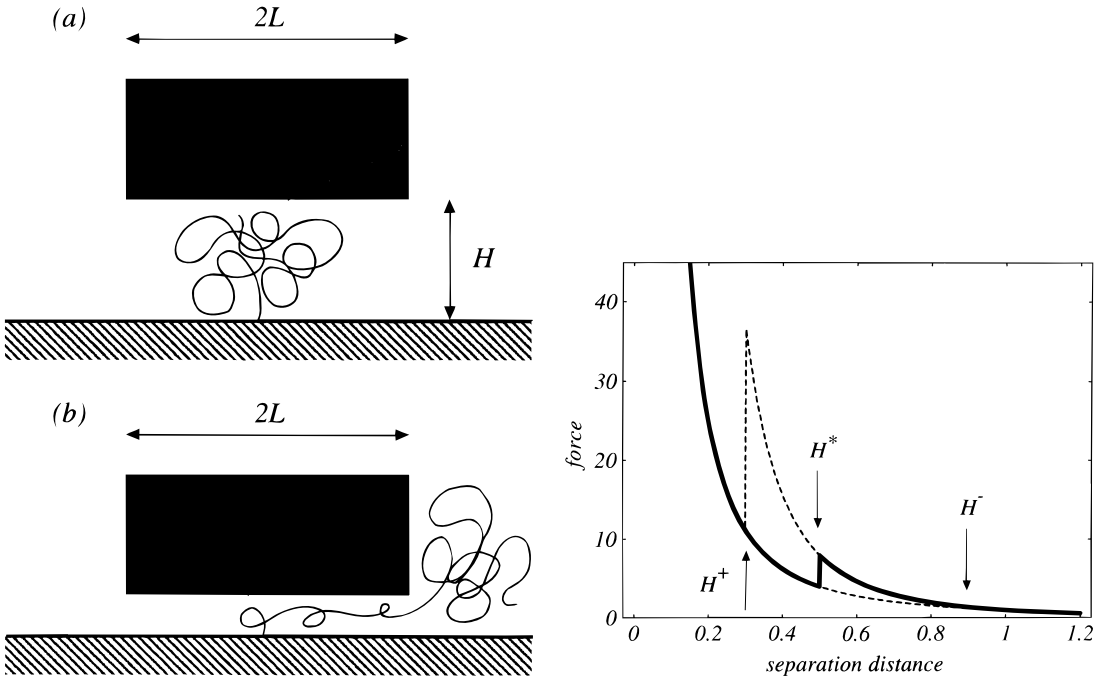


Figure 8: Schematic of AFM stretching a biomolecule, from http://www.astbury.leeds.ac.uk

Domains by AFM", *Science* **276**, 1109-1112, 1997. In this paper, the authors use AFM to study the tension-induced unfolding transition of a giant muscle protein, titin.

- To date, no one has used a polished AFM tip to measure the squashing of a single polymer/biopolymer chain. The idea is that by "sanding" down the point of the AFM tip, you can make a small obstacle that can be used to "flatten" a biopolymer chain that is tethered to a substrate, and measure the force of squashing. However, if the flat end of the AFM tip is comparable in size to the tethered chain, then simple theory predicts that most of the monomers will "escape" from beneath the compressing tip, depending upon the rate at which the tip is lowered. It is relatively easy to explain how this prediction can be made. As the tip is lowered, the chain is confined into a smaller and smaller gap and consequently, its number of possible configurations is diminished and it "loses" entropy. That is, the energy of the chain increases due to entropic confinement. However, at some critical slit distance, the chain can lower its energy by forming a stretched umbilical, from the tether point to the edge of the flat tip, so that most of the chain monomers are escaped from underneath the tip. It does this as the entropic penalty of stretching the umbilical is matched by the gain in entropy of escaped monomers. The squashing force is proportional to the number of monomers trapped underneath the tip and consequently,

upon escape, the squashing from decreases significantly. As the tip is made larger, it takes more time for the chain to "find" the edge of the tip to escape. Indeed, it is possible to do numerical simulation of ideal chain dynamics to predict how this escape transition is affected by the rate of squashing. Such squashing profiles are of interest for a number of reasons. First, one can expect that proteins tethered to a cell are continually being bombarded/impacted by other objects, causing distortions in the shape of the tethered chain. Second, chains tethered onto colloidal surfaces act as "bumpers" to other colloidal particles, stabilising the particles against irreversible aggregation: how these chains deform upon close approach of another surface will determine how effective the chain in stabilising the colloidal solution. If the chain "escapes" from between the surfaces, then it may be an ineffective barrier to aggregation. Colloidal scientists overcome this problem by tethering or grafting many chains in close proximity, rather than isolated chains. We come back to this later.



(a) (b)

Figure 9: (a) Schematic of a polished AFM tip of diameter $2L$ lowered against a substrate containing an end-tethered polymer chain. As the slit or gap distance H decreases, the chain becomes more confined until, at a critical slit distance, H^*, the chain will adopt a low energy configuration that minimises the confinement penalty at the expense of forming a highly stretched umbilical. (b) Prediction of the squashing force as a function of slit distance, H. This prediction is made by comparing the energy of a fully confined chain, , to that of a chain with an escaped chain at a given value of H. That cconfiguration of lowest energy is the predicted configuration and the force required to squash is found by differentiating the energy with respect to H. Reproduced from Guffond, M.C., Williams, D.R.M., Sevick, E.M., *Langmuir* **13**, 5691-5696 (1997).

2.3.2 Optical Tweezers

Optical Tweezers (OT) is a relatively new device, commercially marketed as a tool for nanotechnologists, biologists and clinicians, providing colloid/cell- sorting and micro-dissection capabilities. In OT, a strongly focused beam of light forms a trap that holds a small object. For objects larger than the wavelength of the light, the trap is a result of the light's refraction through an object having a different index of refraction from the surrounding solvent. If η_1 and η_2 are the indices of refraction of the solvent and transparent object, then the geometry of the refraction at the solvent-object interface is given by Snell's Law:

$$\eta_1 \sin\phi_i = \eta_2 \sin\phi_r \tag{18}$$

where ϕ_i is the angle between the incident ray and interface normal, and ϕ_r is the angle between the refracted ray and interface normal. Rays passing through the trapped object refract through 2 interfaces, and for a spherical object, rays are mirrored about the pole of the sphere, as determined by Snell's law. A ray represents a stream of photons, each of which possess momentum, and the ray's intensity corresponds to the flux of photons along the ray. Consider an incident beam of light of uniform intensity. Before refraction, each incident photon has momentum, $\mathbf{p}^i$ with a component directed parallel to the light beam $p^i_\parallel$ and no momentum component perpendicular to the light beam, or $p^i_\perp = 0$. However, after refracting through the object, photons may have a component of momentum that is perpendicular to the light beam, or $p^r_\perp \neq 0$, with the parallel component diminished $p^r_\parallel < p^i_\parallel$. The loss of the photon's momentum in the $\parallel$-direction is imparted to the object, pushing it "downfield. The gain in a photon's $\perp$-momentum, integrated over all the rays refracted through the object is a net zero, due to the mirror symmetry about the pole and the uniform photon flux. Consequently, in a uniform light field, the object is simply propelled in the direction of the incident light.

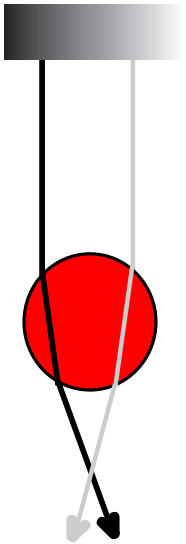


Figure 10: A diagram showing how light imparts momentum to a transparent spherical object, whose diameter is much larger than the wavelength of light, with refractive index larger than the surrounding solvent. The light rays of different intensity refract symmetrically through the object, but there is a difference in photon flux on each side of the object or a net change in photon momentum. This is balanced by the momentum of the object as it moves towards the most intense region of the light beam.

However, if the incident light has a gradient in intensity as shown in figure 10, then the $\perp$-momentum, integrated over all the photons refracted through the object does not vanish and there will be a net change in the $\perp$-momentum , $\Delta p_\perp = p^r_\perp - p^i_\perp > 0$ By the laws of motion, there must be a corresponding momentum imparted to the sphere of equal magnitude but opposite direction. One can show that for an object whose index of refraction is larger than that of the surrounding medium, the object is propelled in the direction of the light gradient, *i.e.*, towards the most intense part of the light beam, as well as downfield.

By placing an objective lens in the light beam, you create a three dimensional gradient field with a point of maximum intensity, the focal point, to which objects are attracted by this "radiation pressure". (Thereby you remove the component of the optical force driving the object downfield.) An optical trap formed from a focussed Gaussian beam (TEM_{00} light mode) is a harmonic trap: the force acting on a colloidal particle positioned $\mathbf{x}$ from the focal point or trap centre is a restoring force

$$\mathbf{f} = -k\mathbf{x}, \tag{19}$$

where k, the trapping constant, is tuned by adjusting the beam intensity. Using a high numerical aperture lens, we can create a trap that is weaker in the focal plane, but much stronger orthogonal to the focal plane. By translating the focal point, objects can be moved, and by increasing the laser power, the beam acts as an optical scalpel, dissecting delicate parts of the object, such as the lining of ovum in clinical IVF procedures. Such trapping systems are becoming highly sought after commercial tools in biological, medical, and clinical laboratories.

However, OT can also be used **to quantitatively measure small forces and this provides unprecedented opportunities to solve a number of important problems in the physical sciences**, ranging from quantifying molecular forces to investigating the stretching of single polymer chains. If there is a small external force acting on the harmonically-trapped particle, then its magnitude is found by simply measuring the particle's displacement from the trap centre. Such harmonic traps have been used recently to measure attractive forces between like-charged colloidal particles, tie knots in single DNA chains, and demonstrate "violations" in the Second Law of Thermodynamics. Moreover, the OT can also be used as a microrheological probe, to measure flow properties of polymer and colloidal solutions at the micron lengthscale (we will discuss more of this in a few weeks).

The physical components of an OT are:

1. **Optical path with objective lens:** The intensity gradient about the focal point is the heart of the OT; the force of the light gradient is proportional to the intensity. Consequently, in order to change k, the trapping constant, you simply alter the intensity or power of your incident light beam. The objective lens that is used to produce the trapping potential can also be used to view or image the trapped object.

2. **Particle position detector:** In order to determine the force acting on the trapped colloid, you simply need to measure its dispalcement from the focal point. One way of doing this is to project an image of the colloidal particle onto a quadrant photodiode detector comprised of four light-sensitive photodiodes arranged into four quadrants. Each quadrant photodiode converts the light intensity over its surface into an electrical signal. By positioning the quadrant photodiode such that its center corresponds to the focal point, then a particle located at the focal point will shadow each of the four quadrant photodiodes equally. However, when the particle is displaced from the focal point, the electrical signal from each of the four quadrants will be out of balance. This balance of 4 signals, (A,B, C, and D) can be calibrated to particle displacement: $x = (A - B)/(C - D)$

3. **Piezoelectric transducers:** Similar to AFM, these transducers are used to translate the stage relative to the light trap or to create flows which impose hydrodynamic or "drag" forces on the colloidal particles.

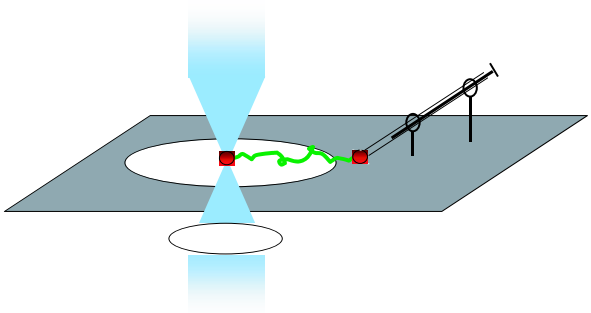


Figure 11: Schematic of how a DNA molecule is stretched in an OT apparatus

In order to measure the stretching force of a a single biopolymer with two end-tethered colloidal beads end-tethered, one simply sucks up one colloidal particle in a stage-mounted micropipette, and positions it a measureable distance from the trap center. This paragraph is a bit of puffery about the new holographic OT system that we are currently building. There are several recent advances in OT, not available in commercial set-ups, that expand the variety of manipulations achievable on small objects [4]. Using a *single* light beam passing though a hologram (which is effectively a phase-only diffractive beam splitter), it is now possible to construct an array of traps in 2 or 3 dimensions, as depicted in figure 12. Depending upon the hologram, traps with different trapping characteristics can be generated, that exert a combination of forces and twist-like torques. For example, a TEM_{00} beam can be converted into helical mode to create an optical "vortex" or "spanner". Particles that are repelled by a harmonic TEM_{00} trap (reflective, adsorbing particles or particles having small index of refraction) are held in the center of the vortex while other particles move in a circular pattern about the vortex centre, driven by the angular momentum of the light. These donut-like traps have been used in fundamental studies of photon spin and momentum [5]. Another example is a "rotator" trap, constructed from the interference of an optical vortex with a planar wave and which exerts a torque. If the hologram is replaced with a computer-addressed SLM or spatial light modulator, a component that dynamically controls the phase-shift and intensity at individual pixel elements, then we can create an array of traps that move or "dance" in 2 or 3 dimensions and dynamically alter the trapping characteristics of each trap. These advances can potentially make difficult OT force measurements far simpler; but more importantly, they also increase the vista of explorations available.

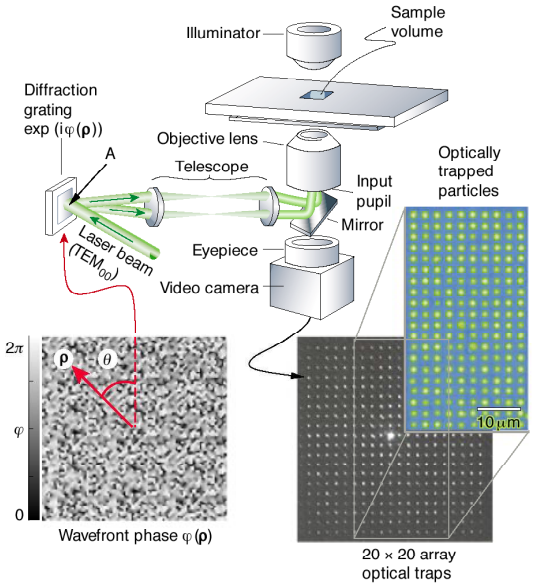


Figure 12: Schematic of an advanced OT set-up using holographic interference to create a dynamic array of traps. Using a computer-controlled Spatial Light Modulator (SLM) in place of the hologram, one can create optical vortices or rotator traps which mpose twist-like torques on particles. The SLM also allows one to dynamically change the array dimensions. From Grier [4].

It is important to recognise that OT and AFM are complementary tools: OT measures forces 0.001-10 pN over micron lengthscales whereas commercial AFMs are capable of measuring forces ranging from 10 - 10,000 pN over nanometer lengthscales. The AFM is effectively a surface measuring device; the OT is foremost a colloidal

technique. OT can measure hydrodynamic forces, forces between colloidal particles

Summary

In this section, we've extended our description of the ideal chain to include thermodynamic predictions of stretching and squashing.

- From the Gaussian distribution (or any distribution) of chain sizes, or any way to count the configurations of a chain, we can evaluate the thermodynamic entropy using Boltzmann's principle.
- For an ideal chain, $\Delta U = 0$ under isothermal conditions and the energy of the chain is entirely entropic. If the chain is deformed under adiabatic conditions, there is no heat exchanged with the surroundings, $\delta q = 0$, and $\Delta U = C_v dT$
- In analogy with the adiabatic compression/expansion of an ideal gas, you can apply first law to predict the adiabatic squashing/stretching of an ideal chain.
- The force of stretching an ideal chain is Hookean; *i.e.*, it is linear with extension.
- The force of compression or squashing on an ideal chain scales as the inverse compression distance, cubed.