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Bending of Actin Filaments.

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Abstract. – We discuss the coupling of bending and torsional degrees of freedom for actin filaments. When a filament is placed in a situation of low symmetry, such as in contact with a surface, actin is shown to develop a spontaneous, non-zero curvature. We discuss the implications of this result on the interpretation of recent experiments which measure the binding modulus of biological filaments.

Actin is an important component of the cytoskeleton of many cells and is one of the principal proteins present in eucaryotes. It is implicated in the generation of movement and as a mechanical support in many biological systems [1]. Under appropriate experimental conditions monomers of actin self-assemble [2] to form filamentary structures which are remarkably like those seen *in vivo*. The structure of actin can be considered as either a tightly pitched single helix or more conventionally but equivalently as a double helix with a pitch of approximately 13 actin molecules; the structure of a filament is also polar with inequivalent ends.

Recently a number of experimental groups have measured the mechanical properties of actin filaments using techniques as varied as light scattering [3], rheology [4], electron microscopy [5] and direct measurement of actin filaments by video optical microscopy [6]. These experiments are often analysed assuming simple bending theory of elasticity [7] in terms of a uniform circular beam fluctuating due to Brownian noise. The effect of these fluctuations is characterised by the persistence length [8], the length over which the tangent vector of the polymer becomes decorrelated. In simple theoretical descriptions of these experiments the energy of a configuration is assumed to be

$$E = \frac{\kappa}{2} \int \frac{1}{R^2(s)} \, \mathrm{d}s \,, \tag{1}$$

with κ a bending elastic constant and $R(s)$ the radius of curvature of the filament at the point s. The persistence length of the filament is then given by $l_\mathrm{p} = \kappa / k_\mathrm{B} T$. When confined to a quasi–

two-dimensional region between microscope slides, a system described by the bending energy (1) can be characterised by the angle θ, the angle of the filament with respect to some arbitrary reference direction. The amplitudes of cosine transform coefficients, θ_q, are determined by equipartition:

$$\langle \theta_q^2 \rangle = \frac{k_B T}{\kappa q^2} \, . \tag{2}$$

Alternatively correlations between tangents to the filament can be characterised by their correlation function

$$\langle \cos(\theta(s) - \theta(0)) \rangle = \exp[-s/2l_p] \, . \tag{3}$$

From a statistical analysis of the configurations of a filament one can deduce the bending constant, κ, using either (2) or (3). There is general agreement that the value of the persistence length of actin filaments is approximately equal to 15 micrometres; however, some groups [6] have found experimental correlation functions which are incompatible with the simple energy (1) which has motivated us to examine the assumptions behind the analysis of these experiments.

Following recent work on the configurational energy of DNA molecules [9], we first remark that there can be a coupling between torsional and bending degrees of freedom. Thus any deduced, macroscopic, elastic constant is a combination of the microscopic bending and torsional constants. In particular, dynamic and static experiments need not give information on the same combination of constants; this is also true of experiments performed in free solution compared with filaments confined in a quasi-two-dimensional geometry. An even more important point discussed in this paper is that we show that in situations of low symmetry the lowest-energy state of an actin filament is no longer linear. In particular many video experiments confine actin filaments between two glass slides (treated with appropriate agents). The equilibrium form of a filament in contact with a surface is curved. We shall now summarise the symmetry arguments that apply to the ground state of actin filaments. We follow this symmetry argument by the solution of a simple perturbative model which displays the essential physics of the phenomenon of symmetry breaking due to an external surface which permits us to estimate the magnitude of the curvature of the filament.

As we have already noted in the introduction, actin filaments are polar, chiral molecules. In solution the chirality of the filament is a strong argument for supposing that the ground state of a filament is linear on length scales which are large compared with the pitch. If we suppose, on the contrary, that a filament is curved, then monomers of actin on the inside of the curvature are in inequivalent positions (they are under compression) compared to other monomers in the filament; the natural equilibrium form of a filament is thus linear. How is this argument modified when we reduce the symmetry of the environment of the filament? We first consider the case of a polar, non-chiral system, then a chiral but non-polar filament to show that both the chiral and polar nature of actin filaments are needed to change the equilibrium form from linear to curved.

A non-chiral but polar filament in contact with a surface is expected to remain linear: reflection in a plane perpendicular to the surface parallel to the filament is a symmetry operation. Thus we expect that the ground state is either linear, or non-unique (which is the case of a filament under external compression where the filament can buckle). Similarly a chiral but non-polar molecule, such as DNA, in contact with a surface has a symmetry operation of rotation by 180 degrees about the normal to the surface which leads to a similar conclusion. We now note that for a chiral molecule a reflection is not a symmetry operation since it interconverts right- and left-handed structures, and that rotation by 180 degrees is not a symmetry operation for a polar object since it reverses the polarity. No symmetry operations exist to characterise the system and we must expect that in general a filament becomes curved. More formally a chiral, polar object has interactions which are a mixture of vector and pseudoscalar in symmetry.

Following recent work on the statistical mechanics of DNA fluctuations [7,9] one can expect in general couplings between twist and bend degrees of freedom. Let us specialise to the case that the filament is forced into intimate contact with a surface. In this situation the equations simplify since the torsion of any planar curve is zero. In addition we wish to model the effect of the interaction of the filament with a surface. We expect that the interaction of filament with a surface should have an attractive non-zero mean, corresponding to van der Waals and more specific interactions between the surface and the proteins plus an oscillating component as the filament rotates with respect to the surface. The simplest model potential respecting the polar and chiral symmetry of the filament is as follows:

$$E = \int \left[\frac{A}{2} \left(\frac{\mathrm{d}\theta}{\mathrm{d}s} - \alpha \cos(\phi) \right)^2 + \frac{A'}{2} \left(\omega - \frac{\mathrm{d}\phi}{\mathrm{d}s} \right)^2 + \lambda \sin(\phi - \phi_0) \right] \mathrm{d}s \,. \tag{4}$$

The parameter λ characterises the amplitude of the fluctuations in the energy of the interaction between the filament and the surface and is thus the source of the twist-bend interaction in this simple Hamiltonian. The term in α represents the oscillation of the filament about the mean position due to the twist of the filament. The fact that the energy is a linear function of both $\sin(\phi - \phi_0)$ and $\cos(\phi)$ breaks the symmetry between s and $-s$. The energy (4) is difficult to treat in general even at quadratic order in the amplitudes, in particular for large values of the parameter λ. We shall proceed by perturbation about the ground state for $\lambda = 0$. We find the variational equations for ϕ

$$A' \frac{\mathrm{d}^2\phi}{\mathrm{d}s^2} - \lambda \cos(\phi - \phi_0) = 0 \,, \tag{5}$$

which can be solved perturbatively in λ to give

$$\phi = \omega s + \frac{\lambda \cos(\phi - \phi_0)}{A' \omega^2} \,. \tag{6}$$

The variational equation for θ then implies that

$$\theta = \alpha \int \cos(\phi) \, \mathrm{d}s \,. \tag{7}$$

From (6) and (7) we deduce that the ground state of the system has a mean curvature given by

$$\dot{\theta}_0 = \frac{\alpha \lambda \sin(\phi_0)}{2A' \omega^2} \,. \tag{8}$$

In particular we recover the expected result of no spontaneous mean curvature in the absence of chirality ($\alpha = 0$) or polarity ($\phi_0 = n\pi$). By considering more general energies one sees in a similar manner that the bending elastic constant is slightly renormalised by the contact between the filament and the surface. Higher-order contributions to the energy also contribute to the bending of the filament but these contributions are numerically small.

We have shown that the equilibrium shape of an actin filament in contact with a surface is no longer linear but is rather uniformly curved. How important are these effects in practice? Since the constants of non-linear elasticity and the variability of the interaction between a surface and the filament are unknown, we shall estimate them from relatively conservative dimensional arguments. The twist elastic constant A' is the most easily estimated and we shall take it equal to $10\,\mu\mathrm{m}\,k_\mathrm{B}T$. The quantity α/ω^2 is equal to the pitch of the helix, 5 nm. The most difficult parameter to estimate is λ. If we assume that the dominant interaction between the filament and the surface is due to van der Waals interactions, we can proceed as follows. The interaction per unit length between a uniform cylinder and a planar surface is given by [10]

$$E_{\mathrm{vdW}} = \frac{A}{12\sqrt{2}} \frac{R^{1/2}}{D^{3/2}}, \tag{9}$$

where R is the radius of the cylinder and D the distance of closest approach between the cylinder and the surface. The Hamaker constant A is expected to be of order of 10^{-20} J in solution. Since the surfaces are often covered with a monolayer of denatured proteins and the experiments are performed at high salt concentrations where the Debye length is equal to 2 nm, we shall take D as about 3 nm. Thus we estimate that the typical interaction between the surface and the filament is of order of $k_\mathrm{B}T/15\,\mathrm{nm}^{-1}$. This seems to be consistent with what is seen in the experiments: filaments with lengths shorter than 1 μm float freely in the solution, longer filaments slide while remaining in contact with the cover slips. We note also that the unbinding transition of a semi-flexible polymer is first order [11] so that once bound to the surface loops are rare in contrast to the case of flexible polymers. This justifies the use of an effective energy confining the filament to a plane. We now estimate the parameter of variability in interaction potentials as being an order of magnitude smaller than its mean value, that is $\lambda = 6k_\mathrm{B}T\,\mu\mathrm{m}^{-1}$. Thus our final estimate of the radius of curvature of the filament is $\dot{\theta}_0 = 2/1000\,\mu\mathrm{m}^{-1}$. This is small compared to typical lengths used in the experimental analysis of actin filament stiffness, however a filament of length 50 μm should be expected to turn through an angle of five degrees because of this effect. This could be seen in careful measurements. These estimates are clearly approximate and could clearly be considerably larger or smaller. If a surface is coated with proteins with a strong specific interaction with actin, these effects could well be magnified though we have not attempted a detailed analysis of this case.

The uniform curvature of a filament would be expected to modify the results of the various methods of analysis differently. In the case of experiments where the whole length of a filament is in contact with a single microscope slide the effect to the curvature is systematic and would lead to curvature in the plot of $\log\langle\cos(\theta(s) - \theta(0))\rangle$.

We expect that

$$\log\langle\cos(\theta(s) - \theta(0))\rangle \approx \frac{s}{2l_\mathrm{p}} - \frac{s^2}{2R_0^2}, \tag{10}$$

with R_0 the radius of curvature of the ground state. Fourier analysis of the shape should lead to an excess in energy in long-wavelength modes. On the contrary in very thin samples a filament which passes between two slides on some specific length scale might be expected to display some non-trivial structure at finite wave vector in the spectrum of fluctuations.

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