

Flexible Polymer-Induced Condensation and Bundle Formation of DNA and F-Actin Filaments

Renko de Vries

Laboratory of Physical Chemistry and Colloid Science and Food Physics Group, Department of Agrotechnology and Food Sciences, Wageningen University, Bomenwegz, 6703 HD Wageningen, The Netherlands

ABSTRACT A simple semi-empirical theory is developed for the ionic strength dependence of the flexible polymer-induced condensation of semiflexible polyelectrolytes such as DNA and F-actin filaments. Critical concentrations of flexible polymer needed for condensation are calculated by comparing the free energies of inserting the semiflexible polyelectrolytes in a solution of flexible polymers, respectively, in their free state, and in their condensed state. Predictions of the theory are compared to experimental data on the condensation of DNA and F-actin filaments induced by the flexible polymer poly(ethylene oxide). The theory also predicts that reentrant decollapse is possible at low ionic strength and high concentrations of flexible polymer, as observed for DNA.

INTRODUCTION

Under some circumstances, semiflexible polyelectrolytes may aggregate or “condense” without completely precipitating out of solution. For example, DNA in very dilute solutions may form condensates of finite size, often with a toroidal geometry (Bloomfield, 1996). Actin filaments may condense to form networks of “bundles” of actin filaments (Tang et al., 1997; Tang and Janmey, 1998). Condensation of semiflexible polyelectrolytes may be achieved either by adding multivalent counterions or other oppositely charged species, or by adding sufficiently high concentrations of “crowding agents” such as uncharged flexible polymers or small globular proteins. These phenomena mimic important aspects of the behavior of these semiflexible polyelectrolytes in vivo. Indeed, the segregation of DNA in bacterial cells has been described in terms of crowding-induced condensation (Odijk, 1998).

The two mechanisms for condensation are antagonistic to each other as far as their ionic strength dependence is concerned (Tang et al., 1997; Tang and Janmey, 1998): condensation by complexation with oppositely charged species is promoted by low ionic strength, crowding-induced condensation is promoted by high ionic strength. It is intuitively clear that crowding-induced condensation should be promoted by high ionic strength: at high ionic strengths, the electrostatic repulsion among the semiflexible polyelectrolytes is screened, and it is easier for the flexible polymer to push the polyelectrolytes together. Nevertheless, there are no theories that quantify the ionic strength dependence of crowding-induced condensation in a consistent way. Here we develop a simple semi-empirical theory for the ionic

strength dependence of crowding-induced condensation of semiflexible polyelectrolytes, specializing to the case of uncharged flexible polymers as the crowding agents. Predictions of the theory are compared to experimental data for flexible polymer-induced condensation of DNA (Frisch and Fesciyan, 1979; Vasilevskaya et al., 1995) and actin filaments (Tang et al., 1997).

Since its discovery (Lerman, 1971), the ψ -condensation of DNA, induced by flexible polymer and high concentrations of monovalent electrolyte, has been studied intensively as a model system for DNA condensation in general (Bloomfield, 1996), both experimentally (Maniatis et al., 1974; Laemmli, 1975; Evdokimov et al., 1976; Frisch and Fesciyan, 1979; Minagawa et al., 1994; Yoshikawa and Matsuzawa, 1995; Vasilevskaya et al., 1995) and theoretically (Frisch and Fesciyan, 1979; Grosberg et al., 1982; Grosberg and Zhestkov, 1986; Vasilevskaya et al., 1995; Ubbink and Odijk, 1995, 1996). Some models for ψ -condensation of DNA do not explicitly include electrostatic interactions (Grosberg et al., 1982; Grosberg and Zhestkov, 1986). Other models do, but predict critical flexible polymer concentrations that are much too low, either at low ionic strength (Frisch and Fesciyan, 1979) or at high ionic strength (Vasilevskaya et al., 1995). Theories for crowding-induced bundle formation of cytoskeletal elements (Madden and Herzfeld, 1993, 1994; Kulp and Herzfeld, 1995) such as actin have so far not included the effect of electrostatic interactions, and hence cannot explicitly predict any ionic strength dependence.

Most theories for DNA ψ -condensation are based on the theory of the coil-to-globule transition that was originally developed for the collapse of flexible polymers in poor solvents (Lifschitz et al., 1978). These theories are based on truncated virial expansions that are valid at low DNA segment concentrations. They are expected to give a rather poor description of the DNA condensates because the DNA concentration inside the condensates can be very high. Ubbink and Odijk (1995, 1996) have worked out detailed theories for toroidal DNA condensates that do not rely on

Received for publication 20 September 2000 and in final form 19 December 2000.

Address reprint requests to Laboratory of Physical Chemistry and Colloid Science, Wageningen University, P.O. Box 8038, 6700 EK Wageningen, The Netherlands. Tel.: +31-317-484561; Fax: +31-317-483777; E-mail: deVries@fenk.wau.nl.

© 2001 by the Biophysical Society

0006-3495/01/03/1186/09 \$2.00

the coil-to-globule transition theory. However, their work mainly deals with the shape of the condensates. It does not directly address the question how much flexible polymer is necessary to achieve condensation, although other aspects of the stability problem are addressed.

To determine whether semiflexible polyelectrolytes will condense or not, we will compare free energies of inserting them into solutions of flexible polymers. More specifically, we will compare the free energy of inserting the semiflexible polyelectrolytes in their “free” state (i.e., freely coiled DNA or isolated actin filaments) with the free energy of inserting them in a condensed state (i.e., DNA condensates, actin bundles). This simplification allows for a much more detailed description of the condensed state that is not necessarily restricted to low densities of the semiflexible polyelectrolyte. The disadvantage is that only the extreme states (“free” and “condensed”) are considered and no, possibly important, intermediate states. Presumably, this is not very serious because it is generally observed experimentally that condensation is discontinuous rather than gradual, at least for individual DNA molecules (Yoshikawa and Matsuzawa, 1995).

First, consider inserting a semiflexible polyelectrolyte in its free state into a solution of uncharged flexible polymers. Because the semiflexible polyelectrolytes are typically much thicker than the uncharged flexible polymers, it makes sense to view the polyelectrolytes as cylindrical obstacles for the flexible polymer coils, and to use depletion theory to calculate the free energy per unit length of inserting the cylinders into the flexible polymer solution. Results for the interaction of excluded volume chains with cylinders have recently been obtained using renormalization-group techniques (Eisenriegler et al., 1996; Hanke et al., 1999). On the basis of these results we propose a simple interpolation formula for the free energy of inserting semiflexible polyelectrolytes in flexible polymer solutions in their free state.

Next, consider the condensed state of the semiflexible polyelectrolytes. Experimentally, it is found (Maniatis et al., 1974; Evdokimov et al., 1976) that, inside condensates, DNA is in a liquid crystalline state, with lattice spacings close to measured spacings in bulk DNA liquid crystals at the same osmotic pressure (Rau et al., 1984; Podgornik et al., 1989, 1994; Strey et al., 1997, 1999). Here we follow Ubbink and Odijk (1995, 1996) in viewing the condensates as small liquid crystals, with a free energy that can be split into a bulk contribution, and a finite size contribution. The bulk contribution is dealt with using Odijk’s theory for hexagonal polyelectrolyte gels (Odijk, 1993a,b). The finite size contribution to the condensate free energy includes a surface term, and an elastic term. The latter is important for DNA condensates: due to the finite size of the DNA condensates, it is usually necessary to curve the DNA molecules in some way or another to fit them into the small condensate volume. As argued for DNA toroids (Ubbink and Odijk, 1995, 1996), it is the balance between surface

energy and elastic energy that determines the condensate shape. Nevertheless, it turns out that the finite size contributions in fact do not greatly affect the critical concentration of flexible polymer needed for condensation. Thus the problem at hand is simplified to calculating the critical concentration for macroscopic phase separation in mixtures of flexible polymer with small amounts of semiflexible polyelectrolyte. This is a much wider problem that may have other applications as well.

THEORY

The reference state for calculating insertion free energies is assumed to be a dilute solution of semiflexible polyelectrolytes in their free state, in the absence of flexible polymer, at the ionic strength of interest. When transferring a semiflexible polyelectrolyte from the reference state into the flexible polymer solution, it can be inserted either in its free state or in its condensed state. Below we calculate the corresponding insertion free energies f_{ins} per unit length of polyelectrolyte.

Inserting a semiflexible polyelectrolyte in its free state

When a semiflexible polyelectrolyte is inserted in its free state, the dominant contribution to the insertion free energy is the free energy of depleting the surroundings of the polyelectrolyte of flexible polymer segments. The depletion layers also cause an effective attraction among neighboring polyelectrolyte segments. However, recent experiments (Vasilevskaya et al., 1995) reveal no significant contraction of the DNA coils before collapse. To simplify the discussion as much as possible, depletion attraction among the polyelectrolyte segments is therefore neglected here when calculating the free energy of inserting the polyelectrolyte in its free state. The insertion free energy is thus approximated by the free energy per unit length of inserting a straight cylinder of radius a . Below, a simple expression for the insertion free energy is derived by interpolating between existing theoretical predictions for various asymptotic regimes. The fact that scaling relations are used implies that most expressions in the present section are only expected to be good to within a multiplicative factor of order unity.

The flexible polymer consists of N segments of length l_K . For simplicity, it is first assumed that the solvent quality for the flexible polymer is very good. Then the segment-excluded volume is $\beta \approx v \approx l_K^3$, where v is the segment volume. Dilute solutions of flexible polymers are characterized by the segment concentration c and by the radius of gyration $R_g \approx l_K N^{3/5}$ of the flexible polymer coils. For c greater than the so-called overlap concentration $c^* \approx N/R_g^3$ the flexible polymers overlap. The resulting semidilute so-

lution is characterized by the mesh size or correlation length $\xi \approx l_K^{-5/4} c^{-3/4}$ (de Gennes, 1979b).

The free energy of inserting a cylinder in a flexible polymer solution sensitively depends on the ratio of the cylinder radius a to the characteristic length scale R_g or ξ of the flexible polymer solution. Thick cylinders, with a radius a much larger than the characteristic flexible polymer length scale, are surrounded by only a thin depletion layer of width R_g , $\xi \ll a$ (see Fig. 1 A). For this case, the insertion free energy is dominated by the osmotic work of depleting the volume to be occupied by the cylinders of flexible polymer segments,

$$f_{\text{ins}} \sim \Pi_f a^2, \quad (1)$$

where Π_f is the flexible polymer osmotic pressure. Note that, in Eq. 1 and in what follows, the symbol f always refers to a free energy per unit length of polyion. For dilute solutions, the osmotic pressure is $\Pi_f = ck_B T/N$. Then, in terms of the weight fraction of flexible polymer $w \approx cl_K^3$,

$$f_{\text{ins}} \sim w \frac{a^2}{l_K^3} k_B T. \quad (2)$$

Semidilute solutions have $\Pi_f \sim k_B T/\xi^3$. For that case,

$$f_{\text{ins}} \sim w^{9/4} \frac{a^2}{l_K^3} k_B T. \quad (3)$$

For thin cylinders, with $a \ll R_g$, ξ (See Fig. 1 B), results for the free energy of insertion were recently obtained by Eisenriegler et al. (1996) and Hanke et al. (1999) for dilute solutions, using renormalization-group methods. The origin of the scaling behavior found by these authors is best understood by relating it to known results for small spheres in solutions of flexible polymers (T. Odijk, personal communication). The free energy of inserting a small inert

sphere of radius a in a flexible polymer coil of gyration radius $R_g \gg a$ is (de Gennes, 1979a)

$$F_{\text{ins}} \sim \left(\frac{a}{R_g} \right)^{4/3} k_B T. \quad (4)$$

Next, we simply view the cylinder as a chain of L/a connected spheres and add up the insertion free energies. For cylinders shorter than R_g that are completely immersed in a flexible polymer coil, this leads to an insertion free energy per unit length of

$$f_{\text{ins}} \sim \frac{a^{1/3}}{R_g^{4/3}} k_B T. \quad (5)$$

For long cylinders in a dilute solution of flexible polymers, the final estimate for the insertion free energy is obtained by multiplying by the volume fraction cR_g^3/N of flexible polymer coils,

$$f_{\text{ins}} \sim \frac{ck_B T}{N} a^{1/3} R_g^{5/3}. \quad (6)$$

This agrees with the renormalization group results of Eisenriegler et al. (1996) and Hanke et al. (1999). In a semidilute solution, the cylinders are fully immersed, and the insertion energy is obtained by replacing R_g in Eq. 5 with the correlation length ξ :

$$f_{\text{ins}} \sim \frac{a^{1/3}}{\xi^{4/3}} k_B T. \quad (7)$$

In terms of the weight fraction of flexible polymer, both Eqs. 6 and 7 reduce to

$$f_{\text{ins}} \sim w \frac{a^{1/3}}{l_K^{4/3}} k_B T. \quad (8)$$

Recently, Sear (1997) proposed an interpolation formula for the free energy of inserting small globular proteins in semidilute solutions of uncharged flexible polymers. In the same spirit, a natural interpolation formula for the free energy per unit length of inserting a cylinder of radius a in a solution of flexible polymers is obtained by simply adding up the asymptotic contributions given by Eqs. 2, 3, and 8. This leads to

$$f_{\text{ins}} \sim \mu_1 w + \mu_2 w^{9/4}, \quad (9)$$

with the following scaling estimates for the numerical prefactors:

$$\mu_1 \sim k_B T \frac{a^{1/3}}{l_K^{4/3}} (1 + (a/l_K)^{5/3} N^{-1}), \quad (10)$$

$$\mu_2 \sim k_B T \frac{a^2}{l_K^3}. \quad (11)$$

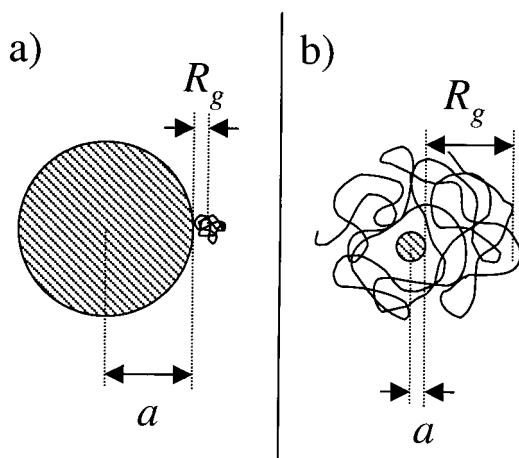


FIGURE 1 Flexible polymer of radius R_g interacting with cylinder of radius a . (A) thick cylinder, $R_g \ll a$; (B) thin cylinder, $R_g \gg a$.

In the respective asymptotic regimes, Eqs. 9–11 reduce to the correct limiting expressions. For thick cylinders, $a \gg R_g, \xi$, they reduce to Eq. 2 for dilute solutions and to Eq. 3 for semidilute solutions. For thin cylinders, $a \ll R_g, \xi$, they reduce to Eq. 8. Obviously, there may be significant errors associated with interpolating between the asymptotic regimes, both between the dilute and semidilute regimes, and between the regimes of thick and thin cylinders. Furthermore, there will be errors associated with our highly simplified flexible polymer model ($\beta \approx \nu \approx l_k^3$). For example, the solvent quality for the depleted flexible polymer segments may not be high enough for them to have a full excluded volume effect. The latter effect is especially pronounced for small globular proteins (Odijk, 1996, 2000). It might be of lesser importance for cylinders because the width of the depletion layer increases significantly in going from a small sphere to a thin cylinder (Eisenriegler et al., 1996; Hanke et al., 1999). We have also neglected the electrostatic image repulsion between the uncharged flexible polymer segments and the highly charged cylinder that might introduce a dependence of the insertion free energy on the ionic strength. However, none of the above effects are expected to change the qualitative behavior of the insertion free energy, being roughly linear in the flexible polymer weight fraction w at low concentrations of flexible polymer, and proportional to the flexible polymer osmotic pressure at high concentrations of flexible polymer. For the special case that the solvent is a theta solvent for the flexible polymer instead of a very good solvent, the first term is expected to remain linear in w (except possibly for logarithmic corrections terms; results for cylinders in flexible polymers under theta conditions follow from known results for thin ellipsoids in ideal Gaussian chains [Jansons and Phillips, 1990; Odijk, 2000]), whereas the exponent of the second term is expected to increase from $3/4$ to 3, because $\Pi_f \sim c^3$ for theta solvents in the semidilute regime.

Inserting a condensate

The free energy of inserting a condensate has two contributions: one is the osmotic work of depleting the volume to be occupied by the condensate of flexible polymer segments, another one is the packing free energy of bringing the polyelectrolyte segments close together, and possibly deforming them to fit in a small volume,

$$f_{\text{ins}} = f_{\text{osm}} + f_{\text{pack}}. \quad (12)$$

The osmotic contribution per unit length of polyelectrolyte is

$$f_{\text{osm}} = \Pi_f V_c / L, \quad (13)$$

where Π_f is the flexible polymer osmotic pressure, V_c is the condensate volume, and L is the polyelectrolyte contour length. As mentioned, semiflexible polyelectrolytes inside

condensates are expected to exhibit liquid crystalline ordering, the polyelectrolyte chains being nearly parallel. This implies that the condensate volume scales as

$$V_c \approx \mu_3 L d^2, \quad (14)$$

where L is the polyelectrolyte contour length, d is the center-to-center distance of neighboring parallel polyelectrolytes in the condensate. The numerical constant is $\mu_3 = 3^{1/2}/2$ for hexagonal packing. Deviations from hexagonal packing in the nematic/cholesteric regime might give a slightly different numerical prefactor, but this is neglected here. Combining Eqs. 13 and 14, the final expression for the osmotic contribution is found to be

$$f_{\text{osm}} = \mu_3 \Pi_f d^2. \quad (15)$$

Experiments are typically performed using poly(ethylene oxide) (PEO) as the uncharged flexible polymer. For the flexible polymer osmotic pressure as a function of polymer weight fraction w , we will here use the empirical expressions of Parsegian et al. (1986). For PEO with a molecular weight $M_w = 6 \cdot 10^3$ at room temperature,

$$^{10}\log(\Pi_f(w)[\text{Pa}]) = 0.61 + 2.795w^{0.21}, \quad (16)$$

where w is in weight percent. Increasing the PEO molecular weight to $M_w = 2 \cdot 10^4$ merely changes the second numerical prefactor to 2.72.

Next, consider the packing contribution to the insertion free energy for condensates. This contribution can be further split into a bulk contribution and a finite size contribution,

$$f_{\text{pack}} = f_{\text{pack,bulk}} + f_{\text{pack,fin}}. \quad (17)$$

In what follows, we will argue that, to a first approximation, the packing free energy is dominated by the bulk contribution, and that the finite size contribution may be neglected. The bulk contribution to the packing free energy equals the free energy of transferring a polyelectrolyte from a dilute solution into a bulk liquid crystalline environment. Except possibly at very small spacings (Rau et al., 1984, Podgornik et al., 1989, 1994) the direct interaction energy $V(d)$ of nearly parallel semiflexible polyelectrolytes on a distance d is dominated by electrostatic repulsion. Electric double layers of neighboring polyelectrolytes will overlap for distances $\kappa(d - 2a) < 2$. For that case, a cylindrical Poisson–Boltzmann cell model would be most appropriate. However, as will be shown a posteriori, we are mainly dealing with nonoverlapping or weakly overlapping electric double layers and distances $\kappa(d - 2a) > 2$. Thus we can use an isolated polyion model. In the far-field Poisson–Boltzmann approximation,

$$\begin{aligned} V(d) &= 2\xi_{\text{eff}}^2 Q^{-1} k_B T K_0(\kappa d) \\ &\approx (2\pi)^{1/2} \xi_{\text{eff}}^2 Q^{-1} k_B T \frac{\exp(-\kappa d)}{(\kappa d)^{1/2}}. \end{aligned} \quad (18)$$

The Debye screening length is $\kappa^{-1} = (8\pi Q n_s)^{-1/2}$, where n_s is the concentration of monovalent electrolyte, $Q = e^2/\epsilon k_B T$ is the Bjerrum length, e is the elementary charge, and ϵ is the solvent permittivity. At room temperature, $Q \approx 0.7$ nm, and $\kappa^{-1} \approx 0.3 n_s^{-1/2}$ nm, when n_s is in molar. The effective dimensionless charge density parameter ξ_{eff} is calculated using the approximate solution to the cylindrical Poisson–Boltzmann equation of Philip and Wooding (1969).

For both DNA and actin, we have numerically tabulated the Philip and Wooding values of ξ_{eff} for a great number of salt concentrations, setting the DNA linear charge density to 1 e per 0.17 nm, the DNA radius to $a = 1.2$ nm, the actin linear charge density to 1 e per 0.25 nm (Tang and Janmey, 1996) and the actin radius to $a = 4$ nm. It was found that the tabulated ξ_{eff} values for DNA could be accurately fitted by a third-order polynomial in the salt concentration. For $0.05 \text{ M} < n_s < 1 \text{ M}$,

$$\xi_{\text{eff}} \approx 1.95 + 30.0n_s + 26.1n_s^2 + 4.9n_s^3, \quad (19)$$

where n_s is again in molar. For actin, it was found to be more expedient to fit the logarithm of the tabulated ξ_{eff} values to a third-order polynomial in the salt concentration. For $0.04 \text{ M} < n_s < 0.18 \text{ M}$,

$$\ln \xi_{\text{eff}} \approx 1.405 + 38.56n_s - 131.83n_s^2 + 255.954n_s^3. \quad (20)$$

At spacing substantially larger than the Debye screening length, thermal shape fluctuations or undulations start to contribute to the total osmotic pressure of liquid crystalline gels of semiflexible polyelectrolytes (Podgornik et al., 1989; Odijk, 1993a,b). Here we use the Odijk, (1993a,b) theory that includes these undulations on a self-consistent field level. Including Gaussian undulations of root-mean-square amplitude u , the total electrostatic interaction energy per unit length of polyelectrolyte in the liquid crystalline environment is

$$f_{\text{el}} = \frac{n_n}{2} V(d) \frac{\exp[\frac{1}{2}(\kappa u)^2]}{1 + \frac{1}{2}\kappa u^2/d}. \quad (21)$$

The (average) number of nearest neighbors of any given chain is n_n , hence there are $n_n/2$ pair interaction per polyelectrolyte strand. The exponential factor multiplying the bare interaction energy, $n_n V(d)/2$, represents the enhancement of the electrostatic repulsion due to thermal undulations of amplitude u . This factor very much favors small undulation amplitudes. In contrast, the configurational entropy f_{entr} of the semiflexible chains favors large undulation amplitudes:

$$f_{\text{entr}} = c \frac{k_B T}{\lambda}. \quad (22)$$

The deflection length, $\lambda = u^{2/3} P^{1/3}$, is the characteristic wavelength of undulations of confined semiflexible chains

(Odijk, 1983). The numerical constant in Eq. 22, $c \approx 3/2^{8/3}$ (Ubbink and Odijk, 1999). Because $\lambda \sim P^{1/3}$, the deflection length may be relatively small, and undulations may be important, even for very stiff polyelectrolytes such as actin. At the ionic strengths of interest, $n_s > 0.1 \text{ M}$, the DNA persistence length $P \approx 50$ nm is independent of the ionic strength (Hagerman, 1988). There is no consensus yet on the precise value of the persistence length of actin filaments. Here we use a value of $P = 2 \mu\text{m}$ as reported by Käs et al. (1996). Values for the undulation amplitude u are determined self-consistently by minimizing the total bulk contribution to the packing free energy,

$$f_{\text{pack,bulk}} = f_{\text{el}} + f_{\text{entr}}, \quad (23)$$

with respect to u . Within the context of the Odijk theory, the osmotic pressure of bulk liquid crystalline gels of semiflexible polyelectrolytes is given by

$$\Pi_{\text{lc}}(d) = \frac{2ck_B T}{3^{3/2}(\kappa d)u^{8/3}P^{1/3}}. \quad (24)$$

The lattice spacing, d , inside the condensates is calculated neglecting the finite size contribution to the packing free energy, by equating the osmotic pressure of the bulk liquid crystalline gels with the external flexible polymer osmotic pressure,

$$\Pi_{\text{lc}}(d) = \Pi_{\text{f}}(w). \quad (25)$$

Together with Eq. 16, this gives a direct relation between the weight fraction of flexible polymer and the lattice spacing d inside the condensates.

The Odijk theory was originally developed for hexagonal liquid crystals for which $n_n = 6$. As it turns out, final osmotic pressures are not very sensitive to the precise value of n_n . Indeed, using this value, semiquantitative agreement is obtained with the recent experimental DNA osmotic pressures of Strey et al. (1997, 1999) for the entire liquid crystalline regime with no adjustable parameters (Kassapidou and van der Maarel, 1998). Quantitative agreement with the data of Strey et al. is possible over most of the liquid crystalline regime if the effective dimensionless charge parameter is used as an adjustable parameter, but this is not necessary in the present context. For monovalent counterions, osmotic pressure data for DNA liquid crystalline phases show some variation with counterion type (Podgornik, 1989). Again, these variations could be accounted for by allowing the effective dimensionless charge parameter to be used as an adjustable parameter, but this is not necessary in the present semiquantitative treatment. Although the Poisson–Boltzmann equation is known to be less accurate for monovalent electrolyte concentration in excess of 0.1 M, the Odijk theory nevertheless gives correct semiquantitative predictions for the osmotic pressure of liquid crystalline DNA up to high ionic strengths, approaching 1 M (Odijk, 1993). This is because under these conditions,

undulations often play an important role, which decreases the sensitivity of the osmotic pressure to details of the direct interactions. For these reasons, we use the Odijk expression as a general equation of state for liquid crystals of semiflexible polyelectrolytes with monovalent electrolytes for all ionic strengths, and use values for the effective dimensionless charge parameters as predicted by the Philip and Wooding (1969) solution of the cylindrical Poisson–Boltzmann equation. Appreciable concentrations of multivalent counterions generally cause attractive interactions among semiflexible polyelectrolytes that are not captured by the Poisson–Boltzmann equation. Therefore, we only consider strictly monovalent electrolytes. Note that Eqs. 21–24 remain valid in the limit where the contribution of undulations to the total gel pressure is negligible, i.e., for $P \rightarrow \infty$ and for lattice spacings of only a few Debye lengths. This concludes the development of the theory for the bulk contribution to the packing free energy.

Finally, consider the finite size contributions to the condensate insertion free energy. These sensitively depend on the details of the condensate shape and the internal packing of the semiflexible polyelectrolytes. As for the internal packing of the DNA in condensates, there are basically two modes: a uniform distribution of DNA curvature, or a highly localized, “defect-like” distribution of DNA curvature. An example of the former is the often-observed toroidal geometry, an example of the latter could be a thick DNA bundle with “hairpin turns” or some other curvature defect at the ends of the bundle. To simplify the discussion, we restrict ourselves to condensates with a more or less uniform distribution of DNA curvature. Then a typical DNA radius of curvature is simply $R_c \sim V_c^{1/3}$ (Ubbink and Odijk, 1996) and the elastic contribution to the insertion free energy of condensates roughly scales as

$$f_{\text{ins}} \sim \frac{P}{R_c^2} k_B T \sim \frac{P}{L^{2/3} d^{4/3}} k_B T. \quad (26)$$

Similarly, the condensate surface area is estimated as $A_c \sim V_c^{2/3}$ and it follows that an estimate for the contribution of the surface energy to the insertion free energy is

$$f_{\text{ins}} \sim \frac{\sigma d^{4/3}}{L^{1/3}}, \quad (27)$$

where σ is the surface energy of the condensate-flexible polymer interface. If the latter is mainly due to the depletion of flexible polymer segments, $\sigma \sim \Pi_f \xi$ for semidilute solutions (Joanny et al., 1979). For a numerical example, consider the typical case $n_s = 0.5$ M, $L \approx 5 \mu\text{m}$ (T4-DNA), for which the critical PEO concentration for collapse is reported to be $w_c \approx 5\%$ for PEO with $M_w \approx 9 \cdot 10^3$ (Vasilevskaya et al., 1995). From the empirical expression Eq. 16 of Parsegian et al. (1986), we estimate $\Pi_f \approx 3.4 \cdot 10^4$ Pa. This leads to a lattice spacing according to the Odijk theory of $d \approx 5.4$ nm. With these numbers, the bulk con-

tribution to the total condensate insertion free energy is estimated to be $f_{\text{ins}} \approx 0.4 k_B T/\text{nm}$. Using a measured value of the correlation length of the semidilute PEO solution of $\xi \approx 1$ nm (Abbot et al., 1992), the finite size contributions are found to be about two orders of magnitude smaller than the bulk contribution. Hence, for typical DNA condensates, it is expected that the finite size contributions to the packing free energy can be neglected. Therefore the critical PEO concentration for condensates should be close to the critical concentration for macroscopic phase separation in mixtures of PEO and small amounts of DNA. For condensates consisting of a single, short piece of DNA, with a length smaller than about a micrometer, finite size corrections may be expected to be nonnegligible.

For actin bundles, the main finite size contribution to the packing free energy is due to the surface free energy of the bundles. A bundle of length l containing n actin filaments has a surface energy of order $\sigma D l$, where $D \sim n^{1/2} d$ is the bundle diameter. The total contour length of actin filament in the bundle is $L = n l$, whence the contribution to the insertion free energy scales as

$$f_{\text{ins}} \sim \frac{\sigma d}{n^{1/2}}. \quad (28)$$

At a typical ionic strength of $n_s = 0.1$ M, the critical PEO concentration at $M_w = 7.5 \cdot 10^3$ is again about $w_c \approx 5\%$ (Tang et al., 1997). This gives an estimated lattice spacing of $d = 10.9$ nm, and a bulk contribution to the insertion free energy of $f_{\text{ins}} \sim 1.1 k_B T/\text{nm}$. Again assuming $\sigma \sim \Pi_f \xi$ and $\xi \approx 1$ nm, the finite size contribution according to Eq. 28 is expected to be at least one order of magnitude smaller than this for $n > 100$, or bundle diameters larger than about 100 nm. From the available micrographs (Suzuki et al., 1989, 1996; Suzuki and Ito, 1996) it is hard to deduce estimates for typical bundle dimensions. Some pictures clearly show bundles thicker than 100 nm. In any case, here, we will simply proceed assuming that finite size contributions can be neglected for actin bundles as well.

COMPARISON WITH EXPERIMENT

Figure 2 reproduces the available experimental data (Frisch and Fesciyan, 1979; Vasilevskaya et al., 1995) for the ionic strength dependence of the critical PEO concentration for DNA ψ -condensation. The datasets are for PEO molecular weights of $\sim 9 \cdot 10^3$ (Vasilevskaya et al., 1995) and $7 \cdot 10^3$ (Frisch and Fesciyan, 1979) and do not differ significantly. Theoretical predictions for the critical PEO concentration are calculated by solving

$$\mu_1 w + \mu_2 w^{9/4} = f_{\text{pack, bulk}} + \mu_3 d^2 \Pi_f \quad (29)$$

with respect to w . For the flexible polymer osmotic pressure, we use Eq. 16. The right-hand side of Eq. 29 implicitly depends on w through the relation between the lattice spac-

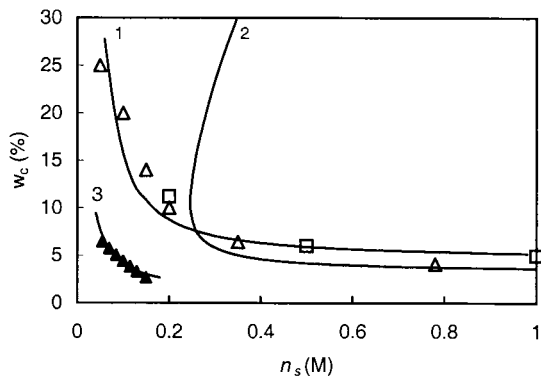


FIGURE 2 Comparison of experimental data with theoretical predictions. Critical concentration w_c of flexible polymer (PEO) needed to condense semiflexible polyelectrolytes, as a function of the concentration of monovalent electrolyte n_s . *Open symbols*, data for DNA (triangles, Vasilevskaya et al., 1995; squares, Frisch and Fesciyan, 1979); *closed symbols*: data for actin (Yang and Janmey, 1997). *Lines*, theoretical predictions: 1. Fitted to DNA data, $\mu_1 = 4 k_B T/\text{nm}$ and $\mu_2 = 50 k_B T/\text{nm}$. 2. example of reentrant decollapse for DNA, $\mu_1 = 7.5 k_B T/\text{nm}$ and $\mu_2 = 22 k_B T/\text{nm}$. 3. Fitted to actin data, $\mu_1 = 8 k_B T/\text{nm}$ and $\mu_2 = 200 k_B T/\text{nm}$.

ing d and the flexible polymer osmotic pressure, Eq. 25. As shown by line 1 in Fig. 2, the theoretical curve fits the data for

$$\mu_1 = 4 k_B T/\text{nm}, \quad \mu_2 = 50 k_B T/\text{nm}. \quad (30)$$

For PEO of much higher molecular weight, Vasilevskaya et al. (1995) find reentrant decollapse at low ionic strengths and very high PEO concentrations. A full comparison with the theoretical predictions for this case is impossible because there is only one data point. Instead, we note that, for different values of the numerical prefactors, reentrant decollapse at low ionic strength and high PEO concentrations is also found in our theory. An example is the solid line 2 in Fig. 2, which is the theoretical prediction for $\mu_1 = 7.5 k_B T/\text{nm}$ and $\mu_2 = 22 k_B T/\text{nm}$.

Next consider PEO-induced bundle formation of actin filaments. The most complete data set is the one by Tang et al. (1997) for PEO with $M_w = 7.5 \cdot 10^3$, which is shown by the filled symbols in Fig. 2. Other authors (Suzuki et al., 1989, 1996; Suzuki and Ito, 1996; Cuneo et al., 1992; Goverman et al., 1996) find very similar critical PEO concentrations for similar PEO molecular weights, but only Goverman et al. (1996) finds critical concentrations that are somewhat larger than those reported by other authors under similar conditions. In comparing the experimental data with theoretical predictions, we neglect the small amounts of divalent counterions that are present in the polymerization buffer. Best agreement with the experimental data was found for

$$\mu_1 = 8 k_B T/\text{nm}, \quad \mu_2 = 200 k_B T/\text{nm}. \quad (31)$$

In agreement with the scaling estimates for the coefficients, $\mu_1 \sim a^{1/3}$ and $\mu_2 \sim a^2$, larger coefficient values are found for actin ($a = 4 \text{ nm}$) than for DNA ($a = 1.2 \text{ nm}$). The second coefficient increases more than the first one, although not by as much as expected on the basis of the scaling estimates.

Although for both actin and DNA, the present theory predicts the right dependence on ionic strength, it does so for values of the numerical coefficients that remain rather poorly understood. The present data is not enough to draw any definite conclusion on the dependence of the coefficients on the polyelectrolyte radius a . In the absence of numerical prefactors, the scaling predictions for the coefficients cannot be used to judge whether the fitted values, and the differences between the fitted values for DNA and actin, are in fact reasonable. Furthermore, according to the scaling predictions, the coefficients hardly depend on the flexible polymer molecular weight, whereas, experimentally, the reentrant decollapse for DNA is observed by increasing the flexible polymer molecular weight. This trend is clearly not captured by the scaling estimates for the coefficients. Summarizing, the comparison with the available experimental data shows that, on the one hand, the present theory captures the generic trends of the dependence on ionic strength, whereas on the other hand, it does a rather poor job in explaining differences between different systems.

As compared to previous theories for DNA condensation (including theories that consider DNA condensation by agents other than flexible polymers, such as those by Post and Zimm, 1982), the main ingredient that allows the present theory to successfully predict the ionic strength dependence of the condensation is that, here, we explicitly include the electrostatic repulsion among parallel polyelectrolytes in the condensates in the Poisson–Boltzmann approximation. Other theories almost invariably use effective interaction parameters for the semiflexible polyelectrolyte segments with an ionic strength dependence that is left unspecified, or assumed to be absent.

DISCUSSION

For both DNA and actin filaments, lattice spacings at the critical PEO concentrations are found to be such that the electric double layers of neighboring polyelectrolytes in the condensates are only weakly overlapping, i.e., $\kappa(d - 2a) > 2$, thus justifying the use of the far-field Poisson–Boltzmann approximation, Eq. 18. Undulations hardly affect the critical PEO concentration for bundle formation of actin filaments: in the low-salt regime considered here, the electrostatic repulsion between actin filaments is still very strong, and undulations are suppressed. The much higher persistence length of actin filaments also reduces the importance of undulations. In contrast, for DNA, the data extends well into the high-salt concentration regime where distances between the DNA strands are many Debye lengths. Coupled with a

much smaller persistence length, this implies that undulations cannot be neglected in calculating the critical PEO concentrations for DNA condensation.

The DNA data clearly shows the existence of two regimes: a high ionic strength regime where the critical concentration is nearly independent of ionic strength, and a low ionic strength regime where the critical concentration sensitively depends on ionic strength. The high ionic strength regime roughly corresponds to the regime of thin cylinders, $R_g, \xi > a$, whereas the low ionic strength regime roughly corresponds to the regime of thick cylinders, $R_g, \xi < a$. In fitting the theory to the experimental data, the coefficient μ_1 mainly determines the critical concentration at high ionic strength, whereas the critical concentration at low ionic strength is more sensitive to the value of μ_2 . The actin data covers only a small intermediate range of ionic strengths, hence the fitted values of the coefficients are somewhat less reliable for that case. Although the different polyelectrolyte parameters, and the different persistence length of actin filaments also contribute to the fact that the critical concentrations are much lower for actin than they are for DNA, the main effect is simply that the free energy of inserting a free actin filament in a PEO solution is much larger than the free energy of inserting a free DNA strand, due to the much larger thickness of the actin filaments.

The reentrant decollapse that is found for DNA for some values of the coefficients is caused by a steep increase of the condensate packing free energy at low ionic strengths: under these conditions, electrostatic repulsion among condensed DNA strands is only weakly screened and hence very strong. Therefore, the lattice spacings at a given osmotic pressure are large. When they are too large, as compared to the flexible polymer correlation length ξ , the flexible polymer may enter the condensate and reentrant decollapse results. Note that, in our highly simplified treatment, we have neglected the possible presence of small amounts of flexible polymer in the condensates that may be important in the regime of reentrant decollapse. Clearly, more data is needed to see whether the present theory also correctly predicts the entire phase diagram under conditions of reentrant decollapse. However, we strongly feel that there is no need to invoke a hypothetical change of the solvent quality for PEO under conditions of reentrant decollapse, as suggested by Vasilevskaya et al. (1995).

Despite the merits of the present theory in semi-empirically describing the available experimental data, it is clear that much more work should be done to achieve a full, testable theory. In view of the poor understanding of the meaning of the fitted coefficient values, it is especially urgent to achieve a more detailed understanding of the free energy of inserting free polyelectrolytes in solutions of flexible, uncharged polymers. On the experimental side, this may be very hard to achieve, but we nevertheless hope that the present work stimulates further experiments in that direction. One question that certainly needs to be addressed

is whether PEO does indeed behave as an inert polymer toward actin and DNA as has been assumed here, because, to mention just one example, PEO reportedly has weak attractive interactions with proteins such as BSA (Abbot et al., 1992). Finally, condensation data for other systems such as, e.g., for TMV or fd viruses, could be helpful in further demonstrating the dominant scaling of the critical flexible polymer concentration for condensation with the polyelectrolyte radius a . Adams and Fraden (1998) have recently published data on mixtures of PEO and TMV, emphasizing bulk phase separation phenomena at a single (low) ionic strength. They have not yet published data at low TMV concentrations as a function of ionic strength so that a comparison for that system is not yet possible.

On the theoretical side, it would be extremely helpful if more detailed results became available for the interaction of flexible polymers with cylinders that are valid over a wider range of flexible polymer concentrations and ratios of a to R_g, ξ . It might also be possible to include the image charge repulsion between the uncharged flexible polymer segments and the polyelectrolytes along the lines of the recent work of Odijk (1997).

Important problems for future work are extensions of the present approach to condensation by other crowding agents such as flexible polyelectrolytes, globular proteins, etc., that carry a charge of the same sign as the semiflexible polyelectrolyte. Also of considerable interest is the ionic strength dependence of the condensation of semiflexible polyelectrolytes for cases where both macromolecular crowding and complexation with oppositely charged species contribute, because that is expected to be the case for DNA condensation and the bundle formation of actin filaments *in vivo*.

I want to thank Theo Odijk for critically reading earlier versions of the manuscript, for suggesting the scaling derivation of Eqs. 5–8, and for many useful discussions. I also would like to thank Cecile Veerman and Robijn Bruinsma for useful discussions.

This work was supported by the Netherlands Research Council for Chemical Sciences (CW) with financial aid from the Netherlands Organisation for Scientific Research (NWO), in the context of the SOFTLINK program "theoretical biophysics of proteins in complex fluids."

REFERENCES

- Abbot, N. L., D. Blankschtein, and T. A. Hatton. 1992. Protein partitioning in two-phase aqueous polymer systems. 3. A neutron scattering investigation of the polymer solution structure and protein-polymer interactions. *Macromolecules*. 25:3932–3941.
- Adams, M., and S. Fraden. 1998. Phase behavior of mixtures of rods (tobacco mosaic virus) and spheres (polyethylene oxide, bovine serum albumin). *Biophys. J.* 74:669–677.
- Bloomfield, V. A. 1996. DNA condensation. *Curr. Opin. Struct. Biol.* 6:334–341.
- Cuneo, P., A. Verzola, and E. Grazi. 1992. "Macromolecular crowding" is a primary factor in the organization of the cytoskeleton. *Biochem. J.* 281:507–512.

- de Gennes, P. G. 1979a. Suspensions colloïdales dans une solution de polymères. *C. R. Acad. Sci. B.* 288:359–361.
- de Gennes, P. G. 1979b. Scaling Concepts in Polymer Physics. Cornell Univ. Press, Ithaca, NY. 69–97.
- Eisenriegler, E., A. Hanke, and S. Dietrich. 1996. Polymers interacting with spherical and rodlike particles. *Phys. Rev. E.* 54:1134–1152.
- Evdokimov, Yu. M., T. L. Pyatigorskaya, O. F. Polyvtsev, N. M. Aki-menko, V. A. Kadykov, D. Ya. Tsvankin, and Ya. M. Varshavsky. 1976. A comparative X-ray diffraction and circular dichroism study of DNA compact particles form in water–salt solutions, containing poly(ethylene glycol). *Nucl. Acids. Res.* 9:2353–2366.
- Frisch, H. L., and S. Fesciyan. 1979. DNA phase transitions: the ψ transition of single coils. *J. Polym. Sci. Polym. Lett. Ed.* 17:309–315.
- Goverman, J., L. A. Schick, and J. Newman. 1996. The bundling of actin with polyethylene glycol 8000 in the presence and absence of gelsolin. *Biophys. J.* 71:1485–1492.
- Grosberg, A. Yu., I. Ya. Erukhimovitch, and E. I. Shakhnovitch. 1982. On the theory of ψ -condensation. *Biopolymers.* 21:2413–2432.
- Grosberg, A. Yu., and A. V. Zhestkov. 1986. On the compact form of linear duplex DNA: globular states of the uniform elastic (persistent) macromolecule. *J. Biomol. Struct. Dyn.* 3:859–872.
- Hagerman, P. J. 1988. Flexibility of DNA. *Ann. Rev. Biophys. Biophys. Chem.* 17:265–286.
- Hanke, A., E. Eisenriegler, and S. Dietrich. 1999. Polymer depletion effects near mesoscopic particles. *Phys. Rev. E.* 59:6853–6878.
- Joanny, J. F., L. Leibler, and P. G. de Gennes. 1979. Effects of polymer solutions on colloid stability. *J. Polym. Sci. Polym. Phys. Ed.* 17:1073–1084.
- Jansons, K. M., and C. G. Phillips. 1990. On the application of geometric probability theory to polymer networks and suspensions, I. *J. Coll. Int. Sci.* 137:75–91.
- Käs, J., H. Strey, J. X. Tang, D. Finger, R. Ezzel, E. Sackmann, and P. A. Janmey. 1996. F-Actin, a model polymer for semiflexible chains in dilute, semidilute and liquid crystalline solutions. *Biophys. J.* 70:609–625.
- Kassapidou, K., and J. R. C. van der Maarel. 1998. Melting of columnar hexagonal DNA liquid crystals. *Eur. Phys. J. B.* 3:471–476.
- Kulp, D. T., and J. Herzfeld. 1995. Crowding-induced organization of cytoskeletal elements. III. Spontaneous bundling and sorting of self-assembled filaments with different flexibilities. *Biophys. Chem.* 57:93–102.
- Laemli, U. K. 1975. Characterization of DNA condensates induced by poly(ethylene oxide) and polylysine. *Proc. Natl. Acad. Sci. USA.* 72:4288–4292.
- Lerman, L. S. 1971. A transition to a compact form of DNA in polymer solutions. *Proc. Natl. Acad. Sci. USA.* 68:1886–1890.
- Lifschitz, I. M., A. Yu. Grosberg, and A. R. Khokhlov. 1978. Some problems of the statistical physics of polymer chains with volume interaction. *Rev. Mod. Phys.* 50:683–713.
- Madden, T. L., and J. Herzfeld. 1993. Crowding induced organization of cytoskeletal elements. I. Spontaneous demixing of cytosolic proteins and model filaments to form filament bundles. *Biophys. J.* 65:1147–1154.
- Madden, T. L., and J. Herzfeld. 1994. Crowding induced organization of cytoskeletal elements. II. Dissolution of spontaneously formed filament bundles by capping proteins. *J. Cell Biol.* 126:169–174.
- Maniatis, T., J. H. Venable, and L. S. Lerman. 1974. Structure of ψ DNA. *J. Mol. Biol.* 84:37–64.
- Minagawa, K., Y. Matsuzawa, K. Yoshikawa, A. R. Khokhlov, and M. Doi. 1994. Direct observation of the coil–globule transition in DNA molecules. *Biopolymers.* 34:555–558.
- Odijk, T. 1983. On the statistics and dynamics of confined or entangled Stiff polymers. *Macromolecules.* 16:1340–1344.
- Odijk, T. 1993a. Undulation-enhanced electrostatic forces in hexagonal polyelectrolyte gels. *Biophys. Chem.* 46:69–75.
- Odijk, T. 1993b. Evidence for undulation-enhanced electrostatic forces from the melting curves of a lamellar phase and a hexagonal polyelectrolyte gel. *Europhys. Lett.* 24:177–182.
- Odijk, T. 1996. Protein–macromolecule interactions. *Macromolecules.* 29:1842–1843.
- Odijk, T. 1997. Depletion around a protein sphere interacting with a semidilute polymer solution. *Langmuir.* 13:3579–3581.
- Odijk, T. 1998. Osmotic compaction of supercoiled DNA into a bacterial nucleoid. *Biophys. Chem.* 73:23–29.
- Odijk, T. 2000. Remarks on the depletion interaction between nanoparticles and flexible polymers. *Physica A.* 278:347–355.
- Parsegian, V. A., R. P. Rand, N. L. Fuller, and D. C. Rau. 1986. Osmotic stress for the direct measurement of intermolecular forces. *Meth. Enzym.* 127:400–416.
- Philip, J. R., and R. A. Wooding. 1969. Solution of the Poisson–Boltzmann equation about a cylindrical particle. *J. Chem. Phys.* 52:953–959.
- Podgornik, R., D. C. Rau, and V. A. Parsegian. 1989. The action of interhelical forces on the organization of DNA double helices: fluctuation-enhanced decay of electrostatic double-layer and hydration forces. *Macromolecules.* 22:1780–1786.
- Podgornik, R., D. C. Rau, and V. A. Parsegian. 1994. Parametrization of direct and soft steric-undulatory forces between DNA double helical polyelectrolytes in solutions of several different anions and cations. *Biophys. J.* 66:962–971.
- Post, C. B., and B. H. Zimm. 1982. Theory of DNA condensation: collapse versus aggregation. *Biopolymers.* 21:2123–2137.
- Rau, D. C., B. Lee, and V. A. Parsegian. 1984. Measurement of the repulsive force between polyelectrolyte molecules in ionic solution: hydration forces between parallel DNA double helices. *Proc. Natl. Acad. Sci. USA.* 81:2621–2625.
- Suzuki, A., M. Yamazaki, and T. Ito. 1989. Osmoelastic coupling in biological structures: formation of parallel bundles of actin filaments in a crystalline-like structure caused by osmotic stress. *Biochemistry.* 28:6513–6518.
- Suzuki, A., and T. Ito. 1996. Polymorphism of F-actin assembly. 2. Effects of barbed end capping on F-actin assembly. *Biochemistry.* 35:5245–5249.
- Suzuki, A., M. Yamazaki, and T. Ito. 1996. Polymorphism of F-actin assembly. 1. A quantitative phase diagram of F-actin. *Biochemistry.* 35:5238–5244.
- Sear, R. P. 1997. Entropy-driven phase separation in mixtures of small colloidal particles and semidilute polymers. *Phys. Rev. E.* 56:4463–4466.
- Strey, H. H., V. A. Parsegian, and R. Podgornik. 1997. Equation of state for DNA liquid crystals: fluctuation enhanced electrostatic double layer repulsion. *Phys. Rev. Lett.* 78:895–898.
- Strey, H. H., V. A. Parsegian, and R. Podgornik. 1999. Equation of state for polymer liquid crystals: theory and experiment. *Phys. Rev. E.* 59:999–1008.
- Tang, J. X., and P. A. Janmey. 1996. The polyelectrolyte nature of F-actin and the mechanism of actin bundle formation. *J. Biol. Chem.* 271:8556–8563.
- Tang, J. X., T. Ito, T. Tao, P. Traub, and P. A. Janmey. 1997. Opposite effects of electrostatics and steric exclusion on bundle formation by F-actin and other filamentous polyelectrolytes. *Biochemistry.* 36:12600–12607.
- Tang, J. X., and P. A. Janmey. 1998. Two distinct mechanisms of actin bundle formation. *Biol. Bull.* 194:406–408.
- Ubbink, J., and T. Odijk. 1995. Polymer-and-salt induced toroids of hexagonal DNA. *Biophys. J.* 68:54–61.
- Ubbink, J., and T. Odijk. 1996. Deformation of toroidal DNA-condensates under surface stress. *Europhys. Lett.* 33:353–358.
- Ubbink, J., and T. Odijk. 1999. Electrostatic-undulatory theory of plectonemically supercoiled DNA. *Biophys. J.* 76:2502–2519.
- Vasilevskaya, V. V., A. R. Khokhlov, Y. Matsuzawa, and K. Yoshikawa. 1995. Collapse of single DNA molecule in poly(ethylene glycol) solutions. *J. Chem. Phys.* 102:6595–6602.
- Yoshikawa, K., and Y. Matsuzawa. 1995. Discrete phase transition of giant DNA dynamics of globule formation from a single molecular chain. *Physica D.* 84:220–227.