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Organized condensation of worm-like chains

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Abstract. – We consider the equilibrium behavior of a wormlike chain of arbitrary length interacting with a spherical organizing center via a short-range attraction. We obtain a rich phase diagram in which a wrapping state is transformed into a multi-leafed ("rosette") structure as the adhesion energy is reduced. By neglecting interactions between the leaves, the statistical mechanics of the "melting" of the rosette reduces to a one-dimensional many-body problem.

Synthetic polymers are often used to control the stability and the aggregation behavior of colloidal suspensions in non-polar solvents [1]. Polyelectrolytes (charged polymers) play a similar role in aqueous solutions [2]. Particularly interesting examples are found in living cells where charged biopolymers like DNA interact with macroions, *e.g.*, proteins. The complexation of DNA with proteins is the basis for the reversible coordinated condensation of long eukaryotic DNA strands of the order of a meter into a highly compacted structure (chromatin) which is confined in a micron-size nucleus [3]. At the lowest point of the hierarchy of "DNA folding", strands are wrapped around organizing proteins (globular complexes of histones) that carry a charge opposite to that of DNA.

The phase behavior of systems of colloidal particles and polymers is rather complex. The problem simplifies significantly if one considers model systems in which a chain interacts with just one "organizing center". We will focus here on the case of a semiflexible chain (like DNA) where the persistence length is of the order of or larger than the particle size (the case of flexible chains was studied by Pincus *et al.* [4]). This problem was first treated by Marky and Manning [5], in whose model the chain interacts with a disk-like particle via a short-range attraction. They found that this system can be either in a bound state where the chain wraps around the particle or in a dissociated state. By changing the binding energy or the persistence length of the chain one can induce an abrupt transition from the bound to the free state. The explicit treatment of electrostatic effects in the complexation of polyelectrolytes with oppositely charged spheres and cylinders was considered in more recent studies [6–8], which also focus on tightly wrapped complexes. On the other hand, in a numerical study by Wallin and Linse [9] of the association of a long polyelectrolyte with an oppositely charged sphere, a more gradual change was observed as the chain stiffness was increased, with one or more loops extending out of the sphere.

In this paper we report on a model study of the finite-temperature conformation of semi-flexible polymers interacting with a spherical organizing center, in an effort to further elucidate the nature of the unwrapping transition. We will demonstrate that chain-ball complexes exhibit open (non-wrapped) structures —"rosette" states— for a wide range of parameters. We solve first for the lowest-energy configurations of the sphere-ball complex and then account for thermal fluctuations. We present a general phase diagram for the wrapped and open structures, and apply the result to a specific system, the DNA-histone complex, at the end of the paper.

We make use of the popular "Worm-Like Chain" (WLC) model [10], which describes the molecule as a semi-flexible tube characterized by two elastic moduli, the bending and torsional stiffnesses. The elastic energy of a WLC of length L can be expressed as

$$E_{\mathrm{elastic}} = \frac{1}{2}\int_0^L \mathrm{d}s \left\{ \kappa \left(\frac{1}{R(s)}\right)^2 + C\left(\frac{\mathrm{d}\theta}{\mathrm{d}s}\right)^2 \right\}. \tag{1}$$

In eq. (1), κ is the bending stiffness and $1/R(s)$ the curvature of the chain at the point s along its contour. The stiffness is normally expressed as $\kappa = k_{\mathrm{B}}T\xi$, with ξ the orientational persistence length of the chain. The torsional angle of the chain is θ and the torsional stiffness is C. The diameter b of the chain is assumed small compared to the diameter D of the spherical organizing center. In addition to the bending contribution, we assume a short-range attraction between the sphere and the chain with binding energy λ per unit length and range $\delta \ll b$. If the adhesion energy $\lambda > \lambda_{\mathrm{c}}$ is large enough, one enters the *wrapping state* with the WLC covering the sphere [6–8, 11]. The critical adhesion energy $\lambda_{\mathrm{c}} \cong 2\kappa/D^2$ can be estimated by comparing the adsorption energy $\lambda\Delta l$ of a short piece of length Δl and its bending energy $2\kappa\Delta l/D^2$.

If $\lambda < \lambda_{\mathrm{c}}$, the WLC will only make point contacts with the organizing center. The energy per adhesive site $\mu \cong \lambda\sqrt{D\delta}$ follows from the length $\sqrt{D\delta}$ of chain that is located within the distance δ from the sphere. The equilibrium number N^* of adhesive sites, and the preferred configurations of the chain, must be obtained by minimization of the energy $E = E_{\mathrm{elastic}} - \mu N$, with E_{elastic} given by eq. (1). We will search first for minima of the elastic energy, neglecting thermal fluctuations.

The rosette state. – This search can be performed systematically by applying the Kirchhoff theory for thin rods [12], which relates stationary points of the WLC energy (*i.e.*, E_{elastic}) to a well-studied classical mechanics problem: the trajectory of a spinning top. Different initial conditions on the Eulerian angles of the top correspond to different stationary points of the WLC energies. The solutions can be characterized in terms of the (topologically conserved) "linking number" (Lk) of the closed strand. Loops of non-zero linking number respond by a combination of twist and spatial distortion (known as "writhing").

We have been able to find solutions of the corresponding Euler-Lagrange equation [13] by imposing the following physical conditions: 1) The WLC must close on itself; 2) it must be possible to inscribe a sphere of diameter D inside the WLC that touches the WLC at N points; 3) there must be no self-intersection of the WLC chain with itself if it is surrounded by a tube of radius b; and 4) the solution must be stable against small perturbations. The ensuing rosette-type configurations can be characterized by the number of loops N. Figure 1 shows an $N = 5$ rosette (computed numerically); it is self-evident that a sphere (or cylinder) can be inscribed in the central hole of the rosette. For each N, we adjusted the linking number of the

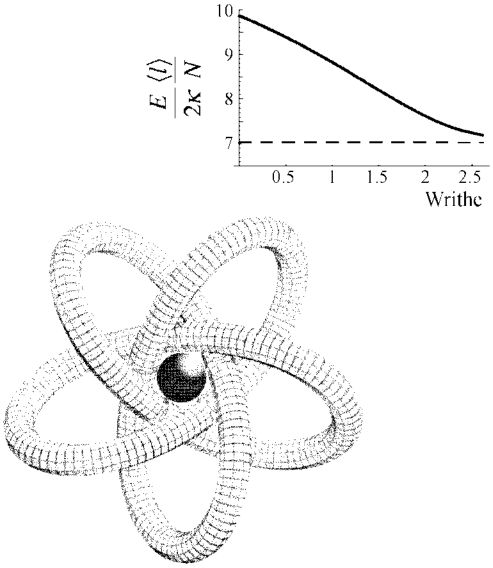

Fig. 1 – Five-leafed rosette with minimal central hole produced by solutions of Euler-Lagrange equations. The spherical organizing center is shown. Inset: elastic energy of the $N = 5$ rosette in dimensionless units ($\langle l\rangle \equiv L/N$) as a function of the degree of writhe. The lowest energy corresponds to the minimal hole size.

loop to minimize the elastic energy. By varying the degree of writhe, a family of solutions was obtained for given N, with different hole diameters. Solutions with the maximum amount of writhe have the smallest central hole diameter as well as the lowest elastic energy. The inset of fig. 1 shows the elastic energy of a loop of length L, in dimensionless units, for the $N = 5$ rosette state, as a function of the degree of writhe.

These solutions are actually *saddle-points* of the WLC energy in that there is a finite subset of all small distortions that lowers the elastic energy of the WLC; the rest either raises the elastic energy or leaves it unchanged (for a detailed discussion we refer the reader to ref. [14]). The key role of the organizing center is here to stabilize the saddle-point solutions and turn them into true minima of the energy. The energy $E_{\min}$ of a minimum-hole rosette depends on L and N as $E_{\min}(N) \cong 2A\kappa N^2/L - \mu N$, with $A = 7.02$ (see inset of fig. 1). A physical interpretation of this result follows from earlier work by Yamakawa and Stockmayer [15] (YS) who showed that a loop of length l, formed by imposing common end-points on a WLC strand, assumes the form of a lemniscate-shaped leaf with an 81-degree apex angle. The elastic bending energy of the leaf is $e(l) = 2A\kappa/l$ and $E_{\min}(N)$ given above just equals $Ne(L/N)$ plus the adhesion energy. We have verified numerically that the leaves of the rosette indeed have apex angles close to 81 degrees. The energy $E_{\min}(N)$ exhibits a minimum as a function of the number N of rosette leaves for $N = N^*$, with $N^* \cong (\mu/k_{\mathrm{B}}T)(L/4A\xi)$. Comparison of the energies of the rosette and the wrapping state shows that the unwrapping into the rosette state takes place at $\mu = \mu_{\mathrm{c}}(1+\varepsilon)$, where $\mu_{\mathrm{c}} = \lambda_{\mathrm{c}}\sqrt{D\delta} \cong 2\xi\delta^{1/2}/D^{3/2}$ denotes the lower bound of local stability of the wrapped state. ε is of the order $(\delta/D)(L/l_{\mathrm{wrap}})$, where $l_{\mathrm{wrap}} \approx D^2/b$ is the length of the wrapped portion of the chain covering the sphere. Hence $\varepsilon \ll 1$ and the wrapping transition is indeed close to $\mu = \mu_{\mathrm{c}}$.

We emphasize that the above results do not depend critically on the assumption that the chain forms a closed loop. In fact, for a chain with open ends we expect the same number N^* of rosette leaves, each again having the shape of a YS-loop. All leaves are now located in a plane, while excluded volume will induce some moderate bending of the loops so that they avoid each other; the energy increase due to this additional bending can be safely neglected.

Note that there is an upper bound $N_{\max}$ for the number of leaves that is of order $(D/b)^{3/2}$. This is the maximal number of contacts, each excluding an area of the order $b\sqrt{Db}$, that can be closely packed on the surface D^2 of the sphere.

Melting the rosette. – To investigate the stability of the rosette against thermal fluctuations, we start from a single, large loop of length L and construct the rosette step by step, by attaching to the organizing center lemniscate-shaped leaves of variable length of the kind examined by YS. The finite-temperature free-energy cost $F(l,\phi)$ of introducing a single leaf of length l and apex angle ϕ into a large strand was computed by YS. Using path integral methods, they found (apart from numerical constants) [15]

$$F(l,\phi)/k_{\mathrm{B}}T = \begin{cases} 2A\dfrac{\xi}{l} + \ln l/\xi + W(\phi) + \ldots, & l \ll \xi\,, \\ \dfrac{3}{2}\ln l/\xi + \ldots\,, & l \gg \xi\,. \end{cases} \tag{2}$$

The function $W(\phi)$ has a minimum when the apex angle of the leaf is approximately 81 degrees. The logarithmic contribution to $F(l,\phi)$ —associated with configurational entropy of the loop, neglecting excluded-volume interaction (Θ solvent)— imposes a free-energy penalty for *large* leaves ($l \gg \xi$). The enthalpic $1/l$ contribution imposes an energy penalty for *small* leaves. For given ϕ, $F(l,\phi)$ as a function of l has a shallow minimum near the persistence length ξ. The total free-energy cost $F_N(\{l_i\})$ of introducing into a large loop an N-leafed rosette *for a fixed distribution* $\{l_i\}$ of leaf lengths is, then,

$$\begin{aligned} F_N(\{l_i\}) &\cong \sum_{i=1}^{N} f(l_i) - \mu N\,, \\ f(l_i)/k_{\mathrm{B}}T &= 2A\xi/l_i + \frac{3}{2}\ln(l_i/\xi)\,. \end{aligned} \tag{3}$$

In eq. (3), $f(l)$ constitutes an interpolation formula between the large and small l limits of $F(l, 81°)$ as given by eq. (2). Note that eq. (3) neglects the twist energy of eq. (1), which we found to be small for large leaf sizes. We neglect furthermore loop-loop collision terms, *i.e.* we assume the case of a Θ solvent. For the case of a good solvent one has inter- and intraloop interactions in the rosette corona that will affect the loop statistics. Note, however, that excluded-volume effects will be most important near the *core*, which is taken into account by allowing at most $N_{\max}$ contact points. The full *free* energy is obtained by performing a Gibbs average over all possible leaf distributions:

$$G(P) = -k_{\mathrm{B}}T\ln\left\{\int_0^\infty \prod_{i=1}^{N_{\max}} \frac{\mathrm{d}l_i}{b}\exp\left[-\frac{1}{k_{\mathrm{B}}T}\left(\sum_{i=1}^{N_{\max}} f(l_i) + P\sum_{i=1}^{N_{\max}} l_i\right)\right]\right\} - \mu N_{\max}\,, \tag{4}$$

with the chain diameter b acting as a short-distance cut-off. We assume in eq. (4) and in the following discussion that $\mu \gg K_{\mathrm{B}}T$ so that $N = N_{\max}$. We have carried out a standard Legendre transformation $G = F + PL$ in order to satisfy (on average) the constraint $\sum_{i=1}^{N} l_i = L$, where P is defined by $L = \mathrm{d}G/\mathrm{d}P$.

Note that $G(P)$ is mathematically identical to the free energy in the constant-pressure ensemble of a one-dimensional many-body system of $N_{\max}$ particles under a "pressure" P confined to a circular track of length L; the particles interact via a "nearest-neighbor pair potential". According to eq. (3), the effective pair potential $f(l)$ is concave (*i.e.*, $\mathrm{d}^2f/\mathrm{d}l^2 < 0$) for inter-particle spacings exceeding a value of order the persistence length ξ, suggesting a possible phase coexistence.

The free-energy equation (4) can be evaluated to be

$$G(P) = N_{\max}k_{\mathrm{B}}T\left\{2\sqrt{\frac{2A\xi P}{k_{\mathrm{B}}T}} + \ln\left(\frac{b/\xi}{\sqrt{\pi/2A}}\right)\right\} - \mu N_{\max}\,, \tag{5}$$

with a corresponding non-linear "tension-extension" curve $P(L) = 2A\xi k_{\mathrm{B}}T(N/L)^2$. Using eqs. (4) and (5), it is straightforward to compute the first and (reduced) second moments of the leaf size distribution: $\langle l\rangle = L/N_{\max}$ and $\Delta l/\langle l\rangle \equiv \sqrt{(l-\langle l\rangle)^2}/\langle l\rangle = \sqrt{L/4AN_{\max}\xi}$. The leaf size grows with chain length in the same manner as the "$T = 0$" solution but the reduced second moment starts to exceed one when the mean leaf size $\langle l\rangle$ exceeds $4A\xi$. We can therefore identify $L/N_{\max} \approx 4A\xi$ as the onset point of *heterogeneity* of the leaf size distribution; the orderly, symmetric rosette is starting to "melt". It must be emphasized though that $G(P)$ is analytic and that there is no true thermodynamic singularity.

This heterogeneous, swollen rosette state is very *fragile*. If we redo the calculation *after cutting the closed loop*, *i.e.*, allowing for two free ends, we recover the result of the closed-loop case but only for $L/N_{\max} \ll AN_{\max}\xi$. In the opposite limit, $L/N_{\max} \gg AN_{\max}\xi$, we find $\langle l\rangle \cong \sqrt{A\xi L/2}$ and $\Delta l/\langle l\rangle \cong (L/A\xi)^{1/4}/2$. The mean leaf size is now small compared to $L/N_{\max}$ and most of the WLC is part of two large chains that emerge from the rosette; this heterogeneity is reminiscent of conventional phase coexistence.

We have summarized our results in a "phase-diagram" of total loop length L *vs.* the adhesion energy μ (see fig. 2) for the case $N_{\max} = 5$. The solid curves indicate transition points between different ground states, namely rosettes with a different number N^* of leaves. The dashed horizontal line indicates the melting point of the rosette with $N = N_{\max}$ at $L/4A\xi \approx N_{\max}$. Note that one encounters phase separation for $L/4A\xi \gg N_{\max}^2$ in the case of an open loop. For small $\mu/k_{\mathrm{B}}T$ (of the order one or smaller) the contact points are not stable thermally (dashed vertical line). For large $\mu/k_{\mathrm{B}}T$ one encounters the wrapping transition (gray bar).

To understand better why there is no phase separation for closed chains (even though $\mathrm{d}^2f/\mathrm{d}l^2 < 0$ at larger separations) we can formally re-compute the free energy for dimensions different from $d = 3$ by replacing the entropy term in eq. (2) by $(d/2)\ln(l/\xi)$. For $d < 4$, the mean loop size is $L/N_{\max}$, while for $d > 4$ we find $\langle l\rangle \cong 8A\xi/(d-4)$ (for large L) and an effective negative *compressibility* of the equivalent many-body system, indicative of phase-separation. This result is suggestive of $d = 4$ acting as an "upper-critical dimension" [16].

Now we consider more closely a specific system, namely the DNA-histone complex (nucleosome [17]). DNA has a diameter of $b = 20\,\text{Å}$ and a persistence length of about $\xi = 500\,\text{Å}$ under standard conditions. It is wrapped around the cylindrical histone core on a helical path of diameter $D = 110\,\text{Å}$. The origin of the attraction between DNA and the octomer is electrostatic (cf. [7]) but can be considered as being short-ranged at physiological salt concentrations, since the screening length ($\leftrightarrow \delta$) $\approx 10\,\text{Å}$. The binding energy per length can be estimated from work by Polach and Widom [18] who found that the nucleosome wrapping state actually represents a *dynamical equilibrium* in which wrapped portions of the DNA strand spend part of their time in a dissociated state. They measured the binding energy λ of DNA to the

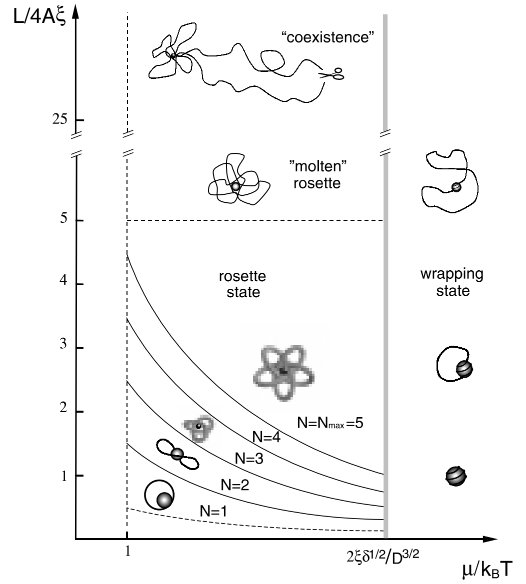

Fig. 2 – Phase diagram as a function of the length L of the loop and the site adhesion energy μ for $N_{\max} = 5$. The wrapping transition is shown as a gray vertical bar. The horizontal dashed line at $L/4A\xi = N_{\max}$ denotes the line above which the rosette leaf structure is heterogeneous due to thermal fluctuations. For large loop lengths, the rosette "phase-separates" if the loop is cut.

nucleosome to be about 1.5–$2k_{\mathrm{B}}T$ per 10 base-pairs (*i.e.* per 34 Å). From this we find the energy of an adhesive site under standard conditions to be $\mu \approx k_{\mathrm{B}}T$.

The *in vitro* phase diagram of mixtures of short DNA strands with single nucleosomal organizing centers has been studied by Yager *et al.* [19]. They found an athermal first-order phase transition as a function of the DNA-histone interaction strength (controlled by changing the salt concentration) from a wrapped state to a dissociated state, consistent with the simple ("all or none") unwrapping transition proposed by Marky and Manning [5]. On the other hand, in the numerical study mentioned above [9], one finds a gradual change of the chain conformations towards more extended complexes by increasing the stiffness of the chain.

These seemingly contradictory observations can be explained by considering the phase diagram in fig. 2. Suppose the DNA strand lengths L are comparable to the persistence length ξ and suppose we vary the adhesion energy μ for *fixed* ξ (and L); then we perform a trajectory in the phase-diagram along a ($L/4A\xi < 1$) horizontal line. For that case fig. 2 predicts a simple "first-order" transition (upon decreasing μ) from a wrapping state to one where there is just a single contact between the chain and the organizing center, consistent with the findings of Yager *et al.* [19] in which unwrapping occurs in a single step upon the addition of salt. A similar horizontal path, but for larger values of $L/4A\xi$, would show a *many*-leafed rosette appearing upon decrease in μ; testing this prediction would involve re-doing the experiments of Yager *et al.* [19] with DNA lengths $L \gg \xi$. Finally, if we decrease ξ for *fixed* (*sub-critical*, $< 2\xi\delta^{1/2}/D^{3/2}$) $\mu/k_{\mathrm{B}}T$, starting from a large value of ξ (*i.e.*, small enough $L/4A\xi$), then the trajectory is a vertical straight line (between $\mu/k_{\mathrm{B}}T = 1$ and $\mu_{\mathrm{c}}/k_{\mathrm{B}}T$) increasing upwards in fig. 2. One encounters a complex sequence of rosette states with increasing N^*, consistent with the simulations of Wallin and Linse [9].

Interestingly, from the earlier numerical estimates for DNA-histone complexation, λ is quite close to λ_c, suggesting that nucleosome-DNA complexes under standard conditions are indeed close to the wrapping transition point. This is consistent with loop formation being postulated as a fundamental step in histone mobility and transcription (see ref. [20] and references therein), and we are presently pursuing a theory of these processes.

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