Raft Instability of Biopolymer Gels*

Itamar Borukhov[1,2] and Robijn F. Bruinsma[2,3]
[1] *Department of Chemistry and Biochemistry, University of California at Los Angeles, Los Angeles, CA 90095-1569, USA*
[2] *Department of Physics, University of California at Los Angeles, Los Angeles, CA 90095-1569, USA*
[3] *Instituut-Lorentz for Theoretical Physics, Universiteit Leiden Postbus 9506, 2300 RA Leiden, The Netherlands*
(October 29, 2004)

Following recent X-ray diffraction experiments by Wong, Li, and Safinya on biopolymer gels, we apply Onsager excluded volume theory to a nematic mixture of rigid rods and strong "$\pi/2$" cross-linkers obtaining a long-ranged, highly anisotropic depletion attraction between the linkers. This attraction leads to breakdown of the percolation theory for this class of gels, to breakdown of Onsager's second-order virial method, and to formation of heterogeneities in the form of raft-like ribbons.

87.15.-v (Biomolecules: structure and physical properties), 61.30.Cz (Molecular and microscopic models and theories of liquid crystal structure), 64.70.Md (Transitions in liquid crystals)

arXiv:cond-mat/0209332 v1 13 Sep 2002

The demonstration by Lars Onsager in 1949 [1] that solutions of long thin rods undergo a first-order phase-transition from an isotropic to a birefringent nematic phase has remained a landmark achievement of theoretical statistical physics. He showed that at the isotropic/nematic transition point the rod volume fraction ϕ is surprisingly low, of the order of the aspect ratio D/L of the rods, D being the rod diameter and L the rod length. Stiff biopolymers provide interesting applications of Onsager theory. In particular, the rod-like filamentous protein actin, which carries many biophysical functions [2], exhibits an isotropic-to-nematic transition [3,4] at a critical volume fraction in approximate agreement with the Onsager criterion [5]. Inside cells, micron-size actin filaments are part of a gel structure, the cytoskeleton, with the filaments cross-linked by reversibly bound proteins like α-actinin. The unusual elastic properties of *in-vitro* actin gels [4,6] have recently been the focus of an intense theoretical effort [7].

In-vitro studies of the sol-gel transition of fixed-length actin filaments are currently interpreted in terms of percolation theory [8], in which linkers are assigned, at random, to rods providing connections to neighboring rods with *no preferred crossing angle*. The sol-gel point, where the real and imaginary parts of the elastic moduli are comparable in magnitude, is identified as the percolation threshold, i.e., the linker concentration for which a connected "path" of linked rods stretches across the sample for the first time. An important validity condition is that the concentration of "native" rod-rod contacts - i.e., the mean number density of rod-rod contacts *before* linkers are added - exceeds the percolation threshold, otherwise the reduction in configurational freedom of the rods imposed by the linkers would lead to phase-separation. The number of native contacts per rod in a dilute isotropic solution of rods is of the order of $(L/D)\phi$ so a rod-linker solution with ϕ of order D/L is expected to exhibit a sol-gel transition that is reasonably well described by percolation theory. The same argument indicates that the structure factor $S(\mathbf{q})$ should resemble that of a pure Onsager nematic.

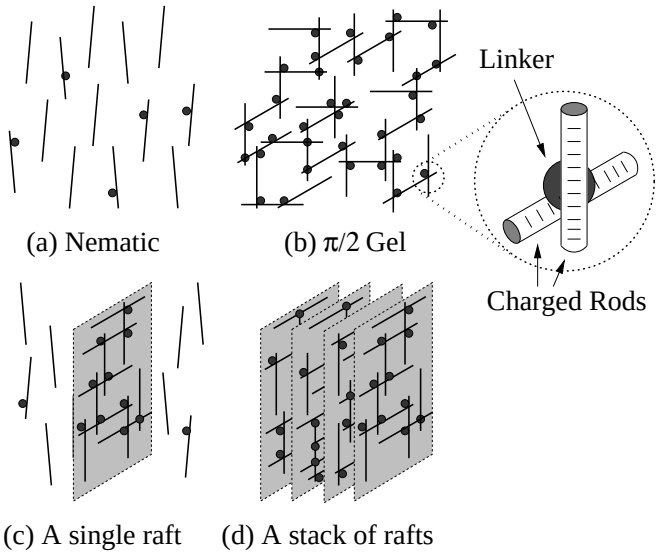

FIG. 1. Mixtures of rigid rods in the nematic phase and "$\pi/2$" linkers display a complex range of structures. (a) At very low linker concentrations and binding energy, excluded volume effects prevent cross-linking. (b) Second-virial theory predicts "$\pi/2$" tetratic or cubatic gels at high linker concentrations. (c) At low linker concentrations a long-range depletion attraction leads to heterogeneous structures in the form of raft-like ribbons along the optical axis. (d) Rafts can stack on top of each other as a result of inter-raft attraction leading to phase separation of the linker-rich stacks from the nematic phase.

*Published in *Phys. Rev. Lett.* **87**, 158101 (2001)

Recent high-precision low-angle synchrotron X-ray studies [9] of the microscopic properties of mixtures of actin filaments and positive divalent counterions report results that are in conflict with these expectations. When divalent counterions are added to a nematic phase of actin filaments, a birefringent gel phase is observed at linker concentrations that depends sensitively on the (average) rod length L, whereas percolation theory would predict that, beyond a certain length, the critical linker concentration is determined only by the rod concentration. In addition, *a harmonic sequence* is found in the structure factor $S(\mathbf{q})$ of the gel phase in the long-wavelength range $1/L \lesssim q \lesssim 1/D$ along directions approximately perpendicular to the optical axis. This is incompatible with the Onsager nematic [10], but it *is* typical of layered structures, such as smectic liquid crystals. The X-ray experiments on actin gels as well as similar results obtained on other biopolymers such as DNA [11] clearly conflict with a simple "Onsager-Percolation" type description.

It is the claim of this paper, that the unusual features of biopolymer gels can be physically interpreted if we allow for the fact that linked biopolymers have preferential crossing angles. First, actin linker proteins are known to impose different crossing angles, depending on their molecular structure. Second, biopolymers like actin or DNA carry a high linear charge density, which allows them to be water soluble. The electrostatic repulsion between two uniformly charged rods linked at a single point depends on the relative angle γ as $1/|\sin\gamma|$ [12], so the optimal crossing angle between two linked biopolymers is, in general, expected to be large. Based on this consideration, we studied - as a simple model for biopolymer gel formation - a dilute mixture of rigid rods with a concentration ρ_p of the order of $1/DL^2$, in the presence of a concentration ρ_l of freely sliding, reversible linkers connecting pairs of rods at $\pi/2$ crossing angles (binding energy ε_0). When $\rho_l = 0$, second-virial theory predicts a first-order phase transition from an isotropic to a nematic phase. It is easy to demonstrate [13] that at non-zero linker concentrations, second-virial theory predicts a first-order phase transition (for $\rho_l \propto \rho_p$) from the nematic phase to a birefringent "$\pi/2$" gel phase with tetragonal symmetry [14] (see Fig. 1b).

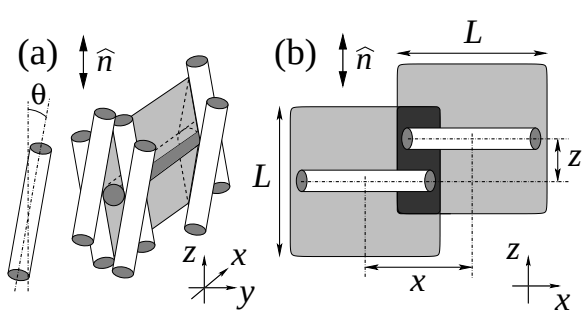

FIG. 2. Schematic view of (a) the depletion volume of a transverse rod in an Onsager nematic and (b) the overlap of two depletion volumes surrounding two transverse rods for $\mathbf{R} = (x, 0, z)$ and $\gamma_{tt} = 0$.

To examine gel formation beyond second-virial theory, we follow what happens when linkers are added, one by one, to the rod solution. A single linker connecting two rods produces a cross-like structure, with the two rods free to slide with respect to each other. It is easy to show that the free energy has a minimum when one arm of the cross extends along the optical axis (the z-direction) with the other arm taken to be the transverse x-direction [15]. As shown in Fig. 2a, the transverse rod creates an anisotropic depletion volume δV_1 for the centers of mass (CM's) of the free rods. This depletion volume depends on the angle γ_{ft} between the axis of a free rod and the axis of the transverse rod by the Onsager result $\delta V_1(\gamma_{ft}) \simeq 2DL^2|\sin\gamma_{ft}|$. The entropic self-energy of the linked cross is computed as the osmotic work $\delta W_1 \simeq \Pi_{\rm osm}\langle\delta V_1(\gamma_{ft})\rangle_\theta$ required to remove the CM's of the free rods from the depletion volume, with $\Pi_{\rm osm}$ the osmotic pressure of the nematic and with $\langle\ \rangle_\theta$ denoting an orientational average. The effect of the arm of the cross along the optical axis is smaller by a factor D/L and will be neglected. Using Onsager theory for the orientational distribution of the free rods, we find:

$$\frac{\delta W_1}{k_BT} \simeq \begin{cases} 2c_I(1+c_I) & \text{(Isotropic, } c_I \lesssim 3.3) \\ (24/\pi)c_N(1-\Delta\theta^2/4) & \text{(Nematic, } c_N \gtrsim 4.5) \end{cases} \tag{1}$$

with $\Delta\theta^2$ being the (small) variance of the polar angle with respect to the optical axis, and $c = (\pi/4)DL^2\rho_p$ the Onsager dimensionless rod concentration. At the isotropic/nematic transition point, the dimensionless free energy cost $\delta W_1/k_BT \simeq 33.2$ assumes a large value independent of the dimensions of the rod, or the rod concentration.

Now add a second linker with a second rod making a $\pi/2$ angle with the optical axis (see example in Fig. 2b). Let the relative distance between the CM's of the two transverse rods be $\mathbf{R}$, let the angle of the second rod with the x direction (i.e., the relative angle) be γ_{tt}, and let $\delta V_2(\mathbf{R}, \gamma_{tt})$ be the excluded volume that is *shared* between the two rods. The entropic free energy cost of the two-rod system will then be

$$\delta W_2(\mathbf{R}, \gamma_{tt}) \simeq 2\delta W_1 - \Pi_{\rm osm}\langle\delta V_2(\mathbf{R}, \gamma_{tt})\rangle_\theta \tag{2}$$

In the limit $R \equiv |\mathbf{R}| \to 0$ and $\gamma_{tt} = 0$, the two rods coincide so $\delta W_2(0,0) \simeq \delta W_1$. On the other hand, for $R \geq L$, there is no shared excluded volume so $\delta W_2(\mathbf{R}, \gamma_{tt}) \simeq 2\delta W_1$. It follows that the two linkers attract each other with a potential energy that has a typical range of order L and a typical binding energy of order δW_1. This effect is related to the well-known *depletion attraction*

[16], the attraction between large objects in a surrounding medium of small objects driven by the increase in configurational entropy as the larger objects coagulate. The difference is that in the present case an effective attraction between small objects (linkers) is generated by an increase in configurational entropy of large objects (rods). Using once again the Onsager orientational distribution, we find that, according to Eq. 2, the linker-linker depletion attraction drops off inversely proportional to the distance between the rods:

$$\frac{\delta W_2(z,\gamma_{tt}) - 2\delta W_1}{\delta W_1} \simeq -a_1 \frac{D}{\Delta\theta|z|} \min\left(\frac{D}{L|\sin\gamma_{tt}|}, 1\right) \tag{3}$$

where z is the separation between the rods along the optical axis and $a_1 \simeq 6/\sqrt{\pi}$. The interaction is highly anisotropic: for small $|z|$, there is a strong torque between transverse rods favoring parallel alignment (i.e., $\gamma_{tt} = 0$). Moreover, the mechanism operates only as long as the CM of the second rod lies below or above the first rod within the two narrow wedges bordered by $|y| \simeq \Delta\theta|z|$ centered on the first rod.

Equation 3 is valid for $D \ll \Delta\theta|z| \ll L$. For small separations $\Delta\theta|z| \ll D$ the potential has a linear dependence on z:

$$\frac{\delta W_2(z,0) - 2\delta W_1}{\delta W_1} \simeq -\left(1 - a_2\frac{\Delta\theta|z|}{D}\right) \min\left(\frac{D}{L|\sin\gamma_{tt}|}, 1\right) \tag{4}$$

with $a_2 \simeq 1/2\sqrt{\pi}$. This result can be understood by noting that for perfectly aligned rods the shared depletion volume of two transverse rods would have a linear dependence on separation [17].

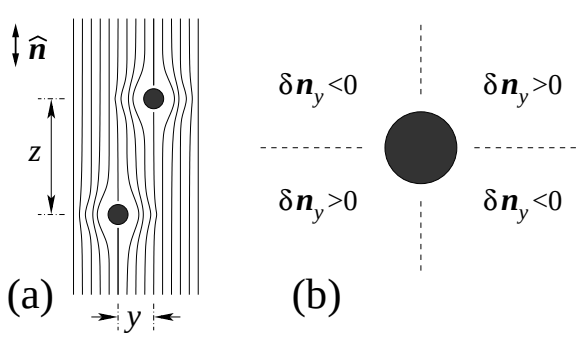

FIG. 3. (a) Schematic view of the long-range depletion interaction between two transverse rods. The continuous lines indicate the director field. (b) The quadrupole symmetry of the distortion zone around a transverse rod.

In the opposite limit of rod separations large compared to the rod length L, the continuum description of a nematic [18] can be used in terms of a locally varying director field $\hat{\mathbf{n}}(\mathbf{r})$ (see Fig. 3a). The perturbation of $\hat{\mathbf{n}}(\mathbf{r})$ far from the fixed rod can be written as $\hat{\mathbf{n}}(\mathbf{r}) \simeq (0, \delta n_y(\mathbf{r}), 1)$, where $\delta n_y \ll 1$. The leading term in the elastic free energy in this geometry is the *splay* term:

$$F_{\text{splay}} = \frac{K_{11}}{2}\int \mathrm{d}^3 r (\nabla\delta n_y)^2 \tag{5}$$

where K_{11} is the splay elastic constant and is equal to $K_{11} \simeq 0.06 DL^4\rho_p^2 k_B T$ for rigid rods [19]. Treating $\delta n_y(\mathbf{r})$ as a variational function and minimizing Eq. 5 leads to the Laplace equation $\nabla^2\delta n_y = 0$. As demonstrated in Fig. 3b, the appropriate solution of the Laplace equation must have the symmetry of a two-dimensional *quadrupole* so $\delta n_y(y,z) = Qyz/(y^2+z^2)^2$. The value of the quadrupole moment Q is determined by the condition that the typical deviation Q/L^2 of $\delta n_y(\mathbf{r})$ for $y^2+z^2 \simeq L^2$ should match the angular deviation D/L of the free rods as obtained from excluded volume arguments (see Fig. 2a). This condition leads to $Q \propto DL$ times a function of $\Delta\theta$. From the electrostatic interaction energy between quadrupoles in two dimensions, $Q^2\cos(4\varphi)/r^4$ (in polar coordinates r, φ), it follows that the interaction energy per unit length between two parallel (infinite) rods must have the form:

$$\frac{F_{\text{splay}}}{Lk_BT} \simeq g(\Delta\theta)\cos(4\varphi)c^2\frac{DL^2}{r^4} \quad r \gg L \tag{6}$$

where the function $g(\Delta\theta)$ includes the dependence on the nematic order parameter. The deformation energy F_{splay} should be matched with the right hand side of Eq. 2 at distances $r \simeq L$ yielding $g \propto 1/c$. It follows from Eq. 6 that the anisotropy of the linker-linker interaction is significantly less pronounced in the regime $r \gg L$. Moreover, the interaction is *repulsive* when the separation vector between the CM's of the rods makes an angle near $\pi/4$ with the optical axis. The various regimes are shown schematically in Fig. 4.

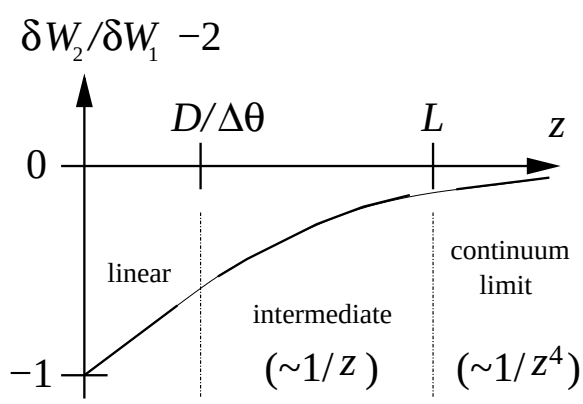

FIG. 4. The depletion attraction between the two rods for $y = 0$.

We now turn to the implication of these results. According to Eq. 1, the characteristic energy scale δW_1 of

the long-range depletion attraction between $\pi/2$ linkers is large compared to the thermal energy k_BT. This implies that percolation theory is not applicable since linkers will cluster even at very low concentrations. Next, second-virial theory is not reliable either since it gives no indication of this form of phase separation. Indeed, the cross-links resemble flat square discs of dimensions $L \times L$ and second-virial expansions are known not be accurate for mixtures of rods and discs [20]. The large value of $\delta W_1/k_BT$ would have another important consequence: unless the linker binding energy is sufficiently high, *crosses simply would not be able to form.* Assuming chemical equilibrium between linkers in solution, linkers adsorbed on isolated chains, and linkers forming a cross-structure, we find that the fraction f of linkers able to form a cross is of order:

$$f \simeq \frac{1}{1 + (\pi/2c)\mathrm{e}^{(\varepsilon_0 - \delta W_1)/k_BT}} \tag{7}$$

This means that cross-links disappear when δW_1 is large compared to ε_0, i.e., when rods are so long that $DL^2 \gg \varepsilon_0/k_BT\rho_p$. This is consistent with the experimental observation that the critical linker concentration for actin gelation rapidly increases with rod length. For an actin solution with $\rho_p \simeq$0.1mM, a linker concentration $\rho_l \simeq$1mM and $\varepsilon_0 \simeq 15k_BT$, the rod length should not exceed a value of order 100nm.

What would be the structure of the clusters? According to Eqs. 3, 4, the depletion attraction between two transverse rods is highly anisotropic at short distances and maximal if the two rods are parallel and located just below each other with a common plane whose normal is perpendicular to the optical axis. Adding additional linkers and transverse rods in the same way produces an elongated ribbon structure resembling a disordered *raft* (see Fig. 1c). The sol-gel transition point should be characterized, not by percolation, but by the formation of elongated, ribbon-like rafts extending through the sample along the optical axis. Because two parallel plates immersed in a nematic attract each other [21] - with a force per unit area that depends on the inter-layer spacing h as k_BT/h^3 for large h - depletion attraction should also operate between *different* rafts, so the ribbons could be several layers thick (Fig. 1d). A multilayer ribbon-raft indeed would have along the transverse direction a structure factor $S(\mathbf{q})$ similar to that of of a smectic, although without long-range order. Along the optical axis, there would have to be a peak in $S(q_z)$ when $2\pi/q_z$ equals the spacing between transverse rods inside the raft, which is indeed present [11].

Our method only applies to a small number of linkers. At finite linker concentration, rafts and ribbons are expected to act as long-lived kinetic intermediates. The actual thermodynamic equilibrium state is expected to be either a $\pi/2$ gel or a biaxial nematic [10,11], in phase-coexistence with linker-poor solution. Numerical simulation methods of the model should be able to address this issue. Next, we did not allow for the fact that linkers may prefer the endpoints of rods nor deviations away from $\pi/2$ for the preferential crossing angle. Finally, actin cytoskeletons *do* appear to resemble $\pi/2$ gels [22] with no evidence of raft formation. Actin filaments inside cells do not have a fixed length however: active polymerization and depolymerization processes are constantly taking place and *in-vitro* experiments of gel formation by such "living" actin filaments would be of great interest.

Acknowledgments: We would like to thank C. Safinya for communicating the results of the X-ray experiments on Actin gels as well as extensive discussions. We would like to thank P. van der Schoot and G.Wong for a critical reading of the manuscript and W. Gelbart, A. Liu, T. Lubensky and C. O'hern for useful discussions.

[1] L. Onsager, *Ann. N.Y. Acad. Sci.* **51**, 627 (1949).
[2] For a review: http://expmed.bwh.harvard.edu/projects/polymer/actin_chapter/index.html by P. A. Janmey, J. X. Tang and C. Schmidt.
[3] C. Coppin and P. Leavis, *Biophys. J.* **63**, 794 (1992); R. Furukawa, R. Kundra, and M. Fechheimer, *Biochem.* **32**, 12346, (1993); P. Das, J. Xu, J. Roy and N. Chakrabarti, *J. Chem. Phys.* **111**, 8240 (1999).
[4] J. Käs, H. Strey, J. X. Tang, D. Finger, R. Ezzell, E. Sackmann and P. A. Janmey, *Biophys. J.* **70**, 609 (1996).
[5] For rod lengths exceeding the persistence length (of order 10μm for Actin) one must replace L with the persistence length. See, e. g., A. R. Khokhlov and A. N. Semenov, *Physica A* **108**, 546 (1981); 112, 605 (1982); Z. Y. Chen, *Macromol.* **26**, 3419 (1993); J. X. Tang and S. Fraden, *Liquid Crystals* **19**, 459 (1995).
[6] B. Hinner, M. Tempel, E. Sackmann, K. Kroy and E. Frey, *Phys. Rev. Lett.* **81**, 2614 (1998).
[7] F. C. MacKintosh, J. Käs and P. A. Janmey, *Phys. Rev. Lett.* **75**, 4425 (1995); R. Everaers, F. Jülicher, A. Ajdari and A. C. Maggs, *Phys. Rev. Lett.* **82**, 3717 (1999); H. Isambert and A. Maggs, *Macromol.* **29**, 1036 (1996); D. C. Morse, *Phys. Rev. E* **58**, 1237 (1998).
[8] M. Tempel, G. Isenberg and E. Sackmann, *Phys. Rev. E* **54**, 1802 (1996); A. Palmer, J. Xu and D. Wirtz, *Rheol. Acta* **37**, 97 (1998).
[9] G. Wong, Y. Li and C. Safinya, UCSB preprint.
[10] P. van der Schoot, private communication.
[11] C. Safinya, private communication.
[12] S. L. Brenner and V. A. Parsegian, *Biophys. J.* **14**, 327 (1974).
[13] R. F. Bruinsma, *Phys. Rev. E*, to be published.
[14] J. Toner, *Phys. Rev. A* **27**, 1157 (1983). This class of liquid crystals were observed in numerical simulations by R. Blaak (unpublished). See also: J. Veerman and D. Frenkel, *Phys. Rev. A* **45**, 5632 (1992).
[15] A second minimum is encountered with two rods tilted by

45° with respect to the optical axis. However, the linker entropy favors the 0° configuration.
[16] S. Asakura and F. Oosawa, *J. Chem. Phys.* **22**, 1255 (1954).
[17] P. van der Schoot, *J. Chem. Phys.* **112**, 9132 (2000).
[18] A similar method was used by P. Poulin, H. Stark, T. C. Lubensky and D. A. Weitz, *Science* **275**, 1770 (1997), to discuss the alignment between spherical inclusions inside a nematic.
[19] R. G. Priest, *Phys. Rev. A* **7**, 720 (1973); J. P. Straley, *Phys. Rev. A* **8**, 2181 (1973).
[20] F. M. van der Kooij and H. N. W. Lekkerkerker, *Phys. Rev. Lett.* **84**, 781 (2000).
[21] A. Ajdari, L. Peliti and J. Prost, *Phys. Rev. Lett.* **66**, 1481 (1991).
[22] P. Janmey, S. Hvidt, J. Lamb and T. Stossel, *Nature* **345**, 89 (1990).