Fascinating Shapes and Structures Due to Entropic Forces

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In most systems that are conventionally studied in physics, the contribution of the internal energy to the free energy dominates over the contribution due to entropy. In contrast, on length scales of the order of micrometers, the internal energy may be comparable to or even much smaller than the free energy arising from entropy. This can happen in systems like polymers and colloids. An important consequence of thermodynamics is that a system adopts a configuration of minimum free energy. Hence in such systems, there is a competition between maximizing entropy and minimizing internal energy. In the extreme case, the system simply goes to the state that maximizes entropy with internal energy having no role to play. These entropic effects are becoming more and more important in soft condensed matter physics and cell biology. In recent times, many experiments have indicated that, in some systems, even an increase in entropy is compatible with the appearance of long range order. This is contrary to the belief that an increase in entropy should always lead to an increase in the degree of disorder. To illustrate this view point we refer to the experiments of Arjun Yodh and his collaborators at the University of Pennsylvania. They studied a system of microscopic spheres of two different sizes suspended in water. Such systems with particles suspended in a liquid are called colloids. Familiar examples are milk, blood, paint and so on. In their experiments, a little salt was dissolved in the water so that the ions could screen the electrostatic repulsion between the spheres. It was observed that after a while the large spheres gradually separated from the solution and arranged themselves as ordered crystals on the walls of the container. Thus surprisingly, a disordered system had evolved into an ordered system, thereby apparently decreasing the entropy. But as a matter of fact, there is no violation of thermodynamics here since actually there is no net decrease in the entropy of the system. The ordering of the large spheres in effect gives more volume for the small spheres to move around, thus increasing their entropy. It is as if the small spheres push the large spheres together in order to increase the space available to themselves. This force, which is purely entropic in origin, is called the depletion force (see the Class Room piece by Supurna Sinha for more details). Initially, the existence of such a force was predicted in 1958 by two Japanese physicists, Asakura and Oosawa. More and more fascinating experiments have been reported since then where the ordering is driven by entropy. In this article, we shall describe two such recent experiments.

The first one by Dinsmore and coworkers considers the entropic effects in vesicles containing large and small spheres suspended

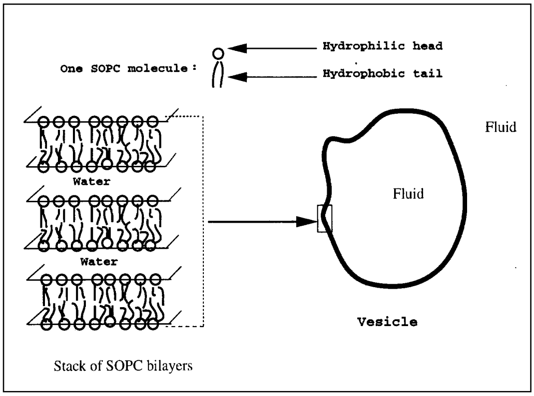
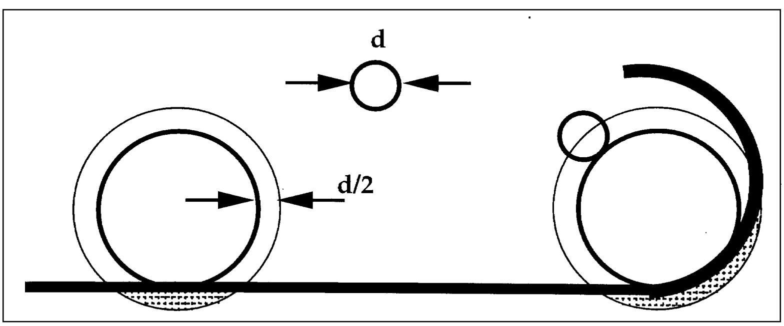


Figure 1. Formation of a vesicle: the wall of the vesicle is made up of many bilayers.

in a liquid. A vesicle is a closed membrane – a bag like structure without any openings, with fluid both inside and outside it, as shown in *Figure 1*. As illustrated in *Figure 2*, the free volume available to the small spheres is more if a large sphere is placed next to the curved surface of the wall of a vesicle than if it is next to its flat region. Also, greater the curvature, higher is the free volume available. Therefore, the large sphere should move towards the curved portions of the wall and occupy regions of higher curvature. However, the curvature of the wall should be less than that of the larger sphere.

The wall of the vesicle is made of 'amphiphilic' molecules. This name is given to molecules which have a portion that likes to be in contact with water and another portion that avoids water. When such molecules are put in water, they arrange themselves to form a vesicle. This is illustrated in *Figure 1*. The wall of a vesicle can be either flexible or rigid. In this experiment, the system was prepared by mixing small and large polystyrene spheres and amphiphilic molecules called stearoyloleoyl phosphatidylcholine (SOPC) in water. The SOPC molecules self-assembled and formed rigid walled vesicles with some of the spheres trapped inside them. The authors first studied a vesicle

Figure 2. The centers of the small spheres can not lie in the annular shell (of thickness equal to the radius of the small spheres: d/2) around the big spheres. This region is called the 'excluded volume' because the small spheres are 'excluded' from here, ie. they are not allowed to enter this region. When the big spheres lie next to the wall, the excluded volumes for the big sphere and the wall overlap (shaded region) and this reduces the total excluded volume. This extra volume becomes available for the small spheres. As shown here, the overlap is more when the sphere is placed next to the curved portion of the wall compared to it being next to the flat portion.



with only one large sphere and no small spheres inside it and found that the free energy of the sphere did not depend on its position; that is, the probability of finding the large sphere was the same everywhere inside the vesicle. Incidentally, the technique that was employed to measure the free energy is rather interesting and worth describing here. Equations of statistical mechanics were used to directly compute the free energy. As the large sphere explored the volume available to it, snapshots of the system were taken at regular intervals of time. Then each picture was divided into a large number of segments and these segments were labeled. At the end, all the pictures were inspected and the number of times the big sphere appeared in a particular segment was counted. The equations of statistical mechanics relate this number to the free energy of the system when the big sphere is in that particular segment. Next, the authors concentrated on a vesicle that happened to have only one big sphere and lots of small spheres inside it. Again the free energy of the system was obtained as a function of the position of the big sphere as it diffused around inside the vesicle. It was found that the probability of finding the large sphere near the wall was higher. In other words, the free energy of the system was a minimum when the large sphere was next to the wall. Also, it was seen that along the wall of the vesicle, the free energy of the system was an absolute minimum when the large sphere drifted to the region of highest curvature. Of course this minimisation is achieved through an increase in the net entropy of the system.

The authors also theoretically worked out the case of a vesicle with a flexible wall where in addition to the effect of entropy described above, the curvature elasticity and the steric repulsion of the wall are also important. Interestingly, they predict that under suitable circumstances, a membrane should curl up around a large sphere.

This conclusion led the authors to propose a rather interesting hypothesis relevant to cell biology. Material is often transported from the cellular interior to the outside by a process called budding. As depicted in *Figure 3*, this involves concentrating the material to be transported at some place near the cell surface and forming a bud on the cell wall which can break off with the relevant materials inside it.

Now we discuss another recent paper by Adams and coworkers who have considered a novel system of a mixture of colloidal rods and spheres suspended in water. In their experiment, for spherical particles they used materials like polyethylene oxide (PEO) or polyethylene glycol (PEG) or polystyrene latex (PS). For rods they used the bacteriophage fd virus. If one takes only spheres suspended in water, the system exhibits an isotropic phase in which there is no positional or orientational order of the constituent particles. This is true even at high sphere concentrations. On the other hand, if only rods are taken, the system exhibits, over a certain concentration range, what is called a

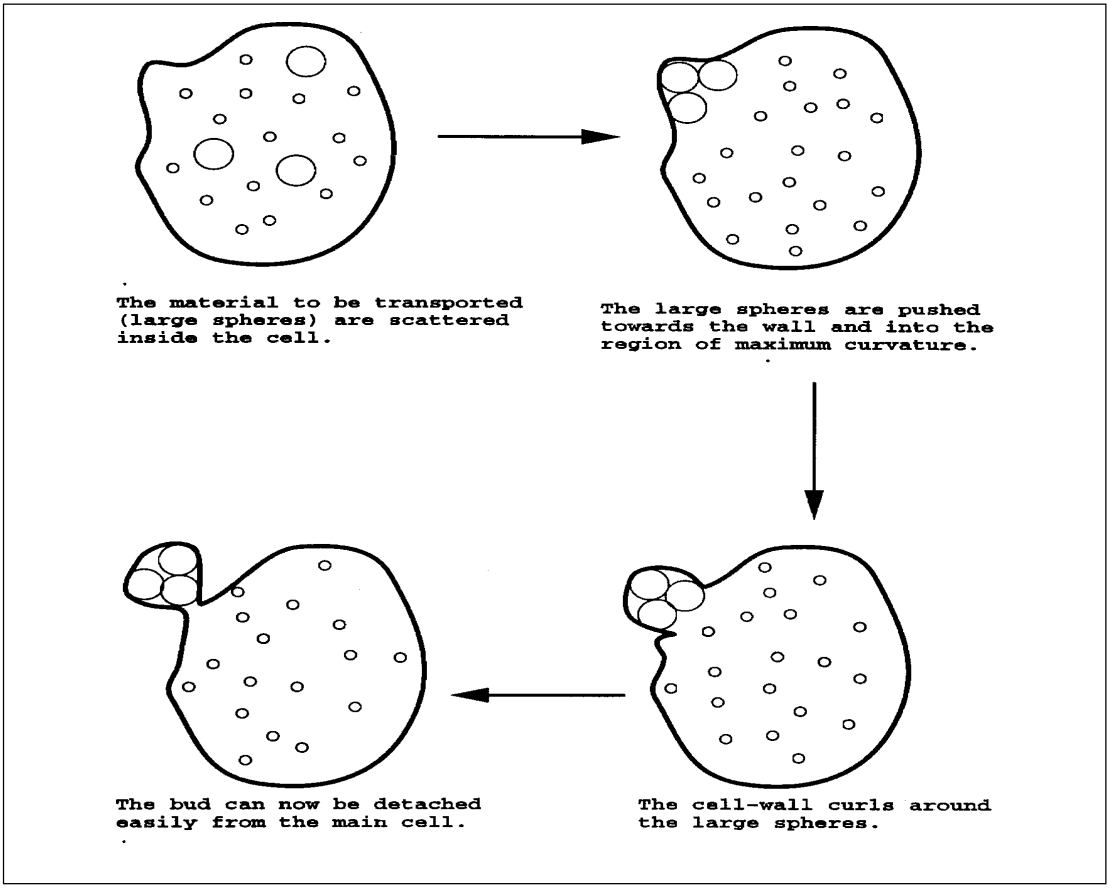


Figure 3. A possible mechanism for transport of materials out of living cells.

nematic liquid crystalline phase. In this phase, the rods align nearly parallel to one another but without any positional order. Thus nematics posses orientational order but no positional order. With further increase in the concentration of the rod-like particles, the system transforms from a nematic to a smectic liquid crystalline phase. In smectics, the molecules arrange themselves in layers. Within each layer the rods are packed normal to the layer but without any positional order. A mixture of rods and spheres in water exhibits more remarkable phases. At low concentrations, the rods and the spheres mix freely, that is, they are completely miscible. At higher concentrations, the mixture transforms to highly ordered complex structures.

A schematic representation of the phase diagram is shown in *Figure 4*. The figure depicts the various phases one obtains either with increasing concentration of spheres or with increasing concentration of rods. For a particular sphere rich concentration, one gets a new phase called the lamellar phase that coexists with the miscible solution. In the

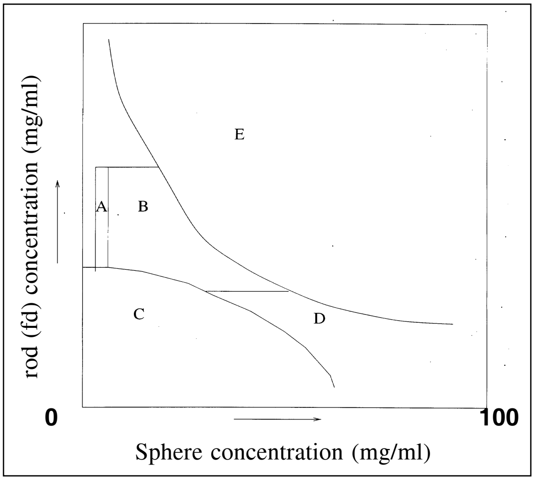


Figure 4. Phase diagram for a mixture of rods (diameter = 6.6 nm, length = 880 nm) and spheres (diameter = 100 nm). A: Columnar phase, B: Lamellar phase, C: Miscible, D: Miscible+Lamellar, E: Disordered Lamellar.

lamellar phase, the layers of rods and spheres arrange themselves alternately as indicated in *Figure 5*. On the other hand, a particular rod-rich solution yields another spectacular phase called the columnar phase. In this phase, the spheres assemble into columns whose diameter is about 3 times that of the individual spheres. These columns are oriented in a direction perpendicular to the long axis of the rods. At this stage, if one increases the sphere concentration, the columns gradually decrease in diameter releasing the spheres and these expelled spheres fill up the gap between the columns. This process continues and finally the system again ends up in a lamellar phase. The lamellar and columnar phases are equilibrium phases and are quite stable. Since care was taken in this experiment to screen the electrostatic repulsion, the authors conclude

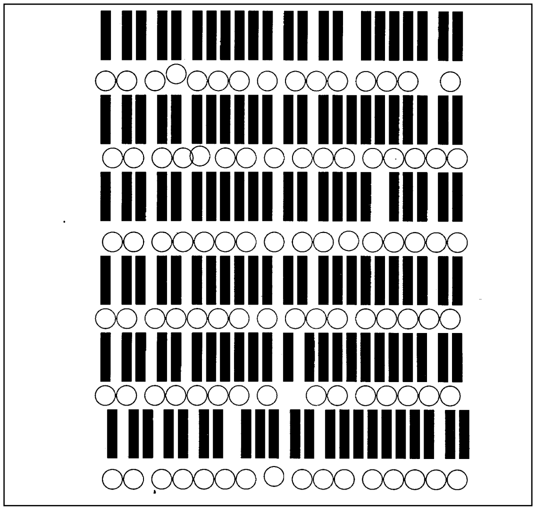


Figure 5. The structure of the lamellar phase.

that entropic forces and packing considerations are the driving forces in the formation of these complex ordered phases.

All this work is certainly a great progress when we remember the 1964 prediction of Lebowitz and Rowlinson which ruled out phase separation even in simple binary hard sphere fluids. It is strange that this prejudice persisted for almost three decades! Now it is beyond any doubt that entropic forces do play a very important role in the formation of complex ordered systems.

Suggested Reading

[1] P D Kaplan, J LRouke, A G Yodh and D Pine. *Phys. Rev. Lett.* 72. 582–585, 1994.

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Adaptive Significance of Circadian Rhythms

Biological Clocks and Darwinian Fitness in Cyanobacteria

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Many physiological and behavioural processes within living organisms are rhythmic, and occur with periodicities of about a day; such biological rhythms are referred to as *circadian* (from the Latin words *circa* = about, *dies* = day). Other such rhythmic physiological and behavioural processes show periodicities of about a month (*circalunar*), or about a year (*circannual*). The periodicities of these biological rhythms closely match those of daily light/dark cycles, or the seasonal cycles of climate, caused by the rotation of the earth around its axis, and around the sun. Marine and intertidal organisms, moreover, are also under the influence of tidal cycles with periodicities of about 12.4 hrs. The most pervasive and widely studied among these biological rhythms are the ones with periods on the order of one day, namely circadian rhythms. These rhythms have been found in organisms ranging from simple unicellular prokaryotic cyanobacteria (blue-green algae) to flowering plants and mammals. In humans, too, many bodily functions, from sleep-wake patterns to core body temperature, exhibit circadian cycles. Flowering in plants shows a circadian pattern, as does foraging activity in many animals. The ubiquitous nature of circadian rhythms strongly suggests that they confer an adaptive advantage to the organism, in terms of adjusting its physiology and behaviour in anticipation of changes in the environment. Yet, there has been little direct experimental evidence in support of such a view, although the adaptive significance of circadian rhythms in nature has been inferred from observations of geographical variation in circadian rhythm parameters, in various species, that is correlated with differences in day length at different latitudes.

In some organisms, like fruitflies and some plants, longevity, growth and developmental rate (which are indirect measures of fitness) have been seen to be higher when individuals were maintained under environments whose periodicities were comparable to the endogenous circadian period of the organism. It was hypothesized that, in such a situation, the biological clock underlying these rhythms is in 'resonance' (no pun intended) with the environmental periodicity and this is beneficial for the physiological well being of the organism. These studies on the possible adaptive significance of circadian rhythms were all carried out on organisms with endogenous periodicities close to 24 hrs. The endogenous periodicity, also called free-running period (FRP), is the periodicity exhibited in a biological rhythm when the organism is kept in an aperiodic environment (e.g. constant light or constant darkness). However, a more rigorous way of determining the adaptive significance of circadian rhythmicity could be to estimate fitness parameters of individuals with altered circadian periodicities that differ considerably from 24 hrs.

In a recent study published in the *Proceedings of the National Academy of Sciences,* USA [1], a group of scientists from USA and Japan has investigated for the first time the relative fitness of individuals with widely differing endogenous periodicities, when reared under environmental light/dark cycles of varying periodicity. In this study, these scientists used various asexual strains of the cyanobacterium *Synechococcus* that exhibit different endogenous periodicities in their luminescence rhythm. In asexual microorganisms, the relative growth of one strain when placed in competition with another is a good measure of reproductive fitness. In this study, two of the four strains of cyanobacteria used expressed wild type endogenous periodicity (FRP=25 hrs), while the other two were mutant strains expressing free-running periodicities of 20 hrs and 30 hrs, respectively. These mutants were derived from the wild type strains by chemical mutagenesis, and carried point mutations within the *kaiC* gene which is one of a cluster of genes, *kaiABC*, that has been shown to play a crucial role in the circadian organisation of *Synechococcus*.

When the strains were grown in pure cultures, no differences in growth rate were seen among the four strains, regardless of whether they were grown in continuous light (LL), light-dark cycles (LD) 12:12 hr, LD 11:11hr, or LD 15:15hr, although the growth rate of all strains was accelerated in LL. This implies that the environmental light/dark cycle *per se,* did not differentially affect the growth rates of these strains. On the other hand, when the different strains were competed against each other in various pair-wise trials, the outcome of competition was seen to be strongly dependent on the endogenous periodicities of the competing strains, and the periodicity of the imposed LD cycle.

These competition experiments done by Ouyang and others were initiated by mixing two strains in equal proportions in a culture, and then tracking their relative proportions over time, in order to assess which strain was the superior competitor (the proportion of the superior competitor in such mixed cultures would be expected to increase over time: this is a standard technique of assessing Darwinian fitness in microorganisms). After being initiated with equal proportions of the two strains, the mixed cultures were exposed to two different LD cycles, LD 11:11hr and LD 15:15hr. When the two wild type (FRP = 25 hrs) strains were competed with each other, they maintained roughly equal proportions for many generations in both types of LD cycles. However, when wild type strains (FRP = 25 hrs) were competed with the short period mutant strain (FRP = 23 hrs), the mutant out-competed the wild type strains in the 22 hr LD (11:11hr) cycle, while the wild type strains out-competed the mutant in the 30 hr LD (15:15hr) cycle. Similarly, when the wild type strains (FRP = 25 hrs) were competed with the long period mutant (FRP = 30 hrs), the wild type strains were the superior competitors in the 22 hr LD cycle, while in the 30hr LD cycle the long period mutant out-competed the wild type strains. When the two mutants (FRP=23 hrs and FRP=30 hrs) were made to compete with each other, the short period mutant was the superior competitor in the 22 hr LD cycle, while the long period mutant out-competed the short period mutant in the 30 hr LD cycle.

These results clearly suggest that the strain whose endogenous periodicity most closely matched the environmental periodicity had a fitness advantage over others in that environment, and was thus favoured by natural selection. Moreover, when the same combinations of strains were grown in mixed cultures in LL, in all cases both strains maintained themselves in equal proportions, indicating that in the absence of a periodic cycle, neither of the two strains had any intrinsic advantage over the other. Although the physiological mechanism by which one strain out-competes the other is not as yet known, the authors speculate that it may be due to competition for limiting resources like light, nutrients and carbon dioxide. Another possible explanation for the results is that these cyanobacterial strains rhythmically secrete diffusible factors that could inhibit the growth of other strains and thus the strain which times its secretion earlier, as a result of differences in its endogenous periodicity, may be at an advantage.

The work by Ouyang and co-workers [1] can be considered as the first step towards rigorously addressing the issue of adaptive significance of circadian rhythms. There are two basic aspects of circadian rhythms, the study of which could give a clear picture of the adaptive significance of these rhythms, namely the free-running nature of these rhythms, and the phase locking to the environmental LD cycles. The outcome of the experiments described here addresses the second point quite clearly. The importance of such phase locking seems to be apparent even in nature, where we often find that different organisms have distinct temporal niches during which they carry out specific activities. The possible adaptive significance of possessing circadian periodicity *per se* is not, however, addressed by the study described here. It has been hypothesized in the past that there may be fitness benefits of possessing a biological clock that are quite independent of the phase locking aspect of circadian rhythms. Such benefits, it is thought, may accrue as a result of being able to synchronise various internal metabolic processes among themselves. Clearly many different lines of work will have to be pursued in order to obtain a full picture of how various aspects of circadian rhythms confer an adaptive advantage to the organisms possessing them; the elegant study of Ouyang and co-workers is a very welcome first step in this direction.

Suggested Reading

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