

Spontaneous Partitioning of Particles into Cellular Structures in a Membrane System

Hajime Tanaka,* Mamoru Isobe, and Jun Yamamoto†

Institute of Industrial Science, University of Tokyo, Meguro-ku, Tokyo 153-8505, Japan

(Received 15 March 2002; published 1 October 2002)

Membranes are often used for material partitioning in biological systems and industry. Here we report a novel physical mechanism of particle partitioning using topological transformation of bilayer membranes. Upon phase separation of a homogeneous sponge phase of a membrane system into a dense sponge and a water phase, we found quite unusual partitioning behavior of particles into the cellular structure of a water phase. The compartments next to one having particles always have no particles, and those next to a compartment having no particles always have particles. We confirm that this partitioning is purely geometrically induced, and thus it may be universal.

DOI: 10.1103/PhysRevLett.89.168303

PACS numbers: 82.70.Uv, 64.70.Nd, 64.75.+g, 82.70.Dd

It is well known that bilayer membranes play quite important roles in biological systems. They can partition a space to form cell structures and control the material transport through pores in membranes. Such stable bilayer membranes are formed by amphiphilic molecules satisfying some conditions on their shape in their aqueous solutions. Membranes further form higher-order structures such as vesicles, lamella, and sponges [1]. A lamellar phase is a highly anisotropic phase having a smectic liquid-crystalline order, while a sponge phase is an isotropic phase in which a water phase is divided by membranes into two identical interconnected regions. This hierarchic structure of membranes is known to reflect that of interactions: The short-range force such as the van der Waals force is responsible for the formation of membranes itself, while the long-range force such as electrostatic repulsion and steric repulsion (the so-called Helfrich interaction [2]) is responsible for the formation and stabilization of lamellar and sponge phases.

In a real biological membrane system, membranes are further mixed with many other components such as polymers, colloids, and proteins. The physics of these membrane complexes has recently attracted much attention, and they are successfully modeled by using surfactant systems for a case that the material is included in membranes [3] and also for a case that the material is confined in a narrow space surrounded by membranes [4–6]. The higher-order structure of membranes is known to exhibit phase transformation by changing either surfactant concentration or temperature [1]. Thus, a natural interesting question associated with this is how the phase transformation affects the spatial distribution of material in a membrane system. In this Letter, we demonstrate a novel phenomenon of spontaneous partitioning of particles into cellular structures, which is induced by phase transformation of the self-organized structure of bilayer membranes.

To model a membrane complex, we add particles (polymer and glass balls) into a membrane system [6]. The membrane systems studied are a mixture of nonionic surfactants, pentaethylenglycol *n*-dodecylether ($C_{12}E_5$) and water and that of triethylenglycol *n*-decylether

($C_{10}E_3$) and water. In these systems, the membranes interact with each other via Helfrich interactions; namely, they are fluctuating in space and colliding with each other, which causes the steric repulsion because of the entropic penalty. Since the Helfrich interaction is quite long range, we can prepare stable hyperswollen lamellar and sponge phases, whose characteristic intermembrane spacing is in the order of sub- μm [7]. These hyperswollen phases make it possible to expand the characteristic size of particles, which can fit in a space between membranes, up to a semimacroscopic length scale, namely, the order of sub- μm . This enables us to directly observe the spatial distribution of particles with optical microscopy. Polymer balls used are monodisperse charged polystyrene latex whose diameters are 107, 175, and 356 nm, while glass particles are monodisperse charged silica particles whose diameters are 80 and 120 nm. Since only balls have surface charges and membranes are electrically neutral, balls interact with membranes only sterically. Furthermore, electrostatic repulsion among balls prevents them from aggregation and helps their homogeneous dispersion in a space between membranes.

The key physical factor of a mixture of membranes and balls is the ratio of intermembrane spacing a and ball diameter d . Here it should be noted that the spacing a in a sponge phase is about $\alpha = 1.4$ times wider than that in a lamellar phase at the same surfactant concentration [8]. Thus, the spacing a of the sponge phase is given by $a = \alpha\delta/\phi$, where ϕ is the membrane concentration and δ is the thickness of a membrane ($\delta \cong 3.8$ nm for $C_{12}E_5$) [7]. We set the ball diameter d to be comparable to the interlamellar spacing. This realizes the homogeneous distribution of balls in the sponge phase. The phase diagram of an aqueous solution of $C_{12}E_5$ with polystyrene latex particles ($d = 107$ nm) is shown in Fig. 1. In our experiments, the temperature of a sample was quickly raised to a certain temperature and the morphology was observed with optical microscopy. When a system passes the coexistence region between a lamellar and a sponge phase, no evident change in the particle distribution is observed. When a temperature reaches the phase-separation

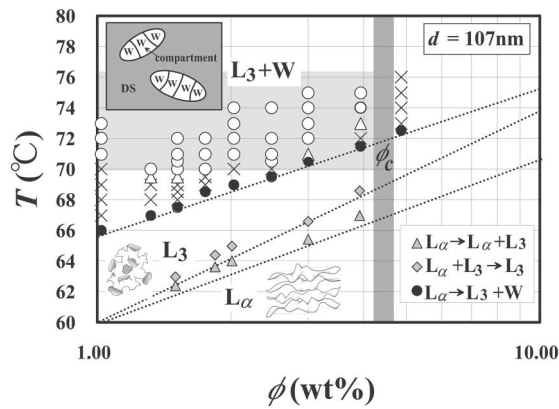


FIG. 1. A phase diagram of a mixture of $C_{12}E_5$ and water including latex particles ($d = 107$ nm), which was determined by the visual observation and the rheological measurement. The phase diagram is not affected by the inclusion of latex particles if their concentration is less than a few wt%. Thus, it is consistent with the phase diagram of the same system without particles previously reported by Strey *et al.* [7]. The results of the check of the occurrence of the particle partitioning are indicated in the figure for mixtures including 0.2 wt% of polystyrene latex particles ($d = 107$ nm). \circ : perfect partitioning; Δ : partial partitioning, \times : no partitioning. The vertical gray line indicates the concentration ϕ_c at which the characteristic length of the sponge a becomes comparable to the particle size d .

temperature T_{PS} , a homogeneous sponge phase separates into a denser sponge phase and a very dilute micellar phase [7]. Since the dilute micellar phase is almost pure water, hereafter we call it the “water” phase. Above T_{PS} , a water phase is nucleated in a homogeneous sponge phase and grows in its size. For a mixture of balls and membranes, a quite surprising phenomenon is observed when a water phase is nucleated in a sponge phase upon heating above T_{PS} . For the case of $a < d$, almost all the balls are expelled from a dense sponge phase and go into a water phase. What surprises us is that a compartment having many balls inside always has empty compartments as its neighbors, as shown in Fig. 2(a). In other words, balls are perfectly partitioned into compartments in an alternative way. This phenomenon indicates that each compartment has a parity, “I” and “O”. Here O means an outside compartment having many balls in it, while I means an inside one having no particles. For the definition of “inside” and “outside,” see Figs. 3 and 4. A type I compartment can never have type I ones as its neighbors. Only $IOIOIO$ -type alternative configuration is allowed. This strongly suggests that there is an underlying symmetry-breaking transition, which is responsible for such a peculiar way of the partitioning of particles into compartments (or cells).

We studied in detail under what conditions this unusual phenomenon is observed. The results (those for particles of 107 nm are shown in Fig. 1) indicate that it occurs only when the dense sponge phase coexisting with

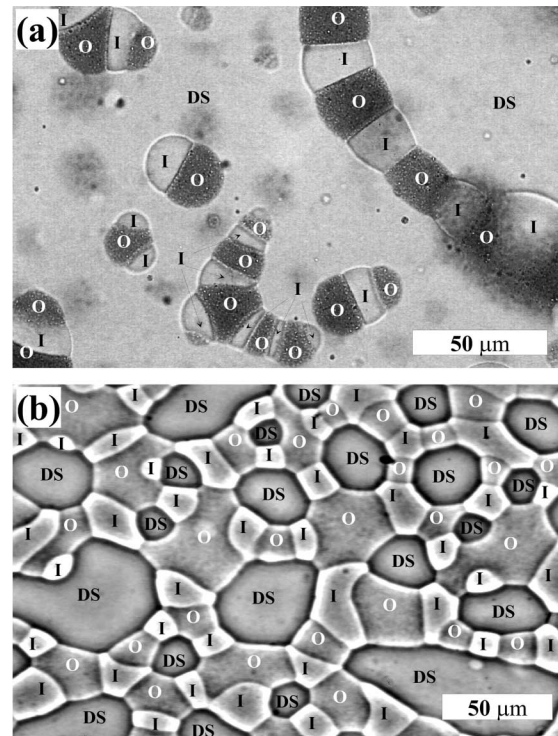


FIG. 2. (a) Pattern observed under optical microscopy in an aqueous solution of $C_{12}E_5$ (2.5 wt% $C_{12}E_5$) and latex particles (0.2 wt% latex) in the coexistence region of a dilute micellar (water) and a dense sponge phase (DS) at $T = 72.0$ °C. For this concentration of $C_{12}E_5$, the intermembrane spacing a is about 200 nm for a lamellar phase, while it is about 280 nm for a sponge phase. The particle diameter d is, on the other hand, about 107 nm. The other experimental conditions are completely the same as those in Fig. 3. The only difference is the existence of particles. In the figure the darker compartments are filled with particles, which are moving vigorously by Brownian motion. Coalescence between droplets of a water phase occurs only between compartments of the same type. (b) Pattern observed in an aqueous solution of $C_{12}E_5$ (2.0 wt% $C_{12}E_5$) and silica particles (2.0 wt% silica) at $T = 74.0$ °C. The diameter d of silica particles is about 80 nm. Note that in (a) a minority phase is the water phase, while in (b) it is the dense sponge one, reflecting the difference in the location in the phase diagram between them.

the water phase has the concentration higher than a critical one $\phi_c(d)$, or the intermembrane spacing smaller than $a_c(d)$. We also determine a_c for cases of $d = 175$ nm and $d = 356$ nm by the same method. From these results, we found a very simple relation between a_c and d , namely, $a_c = d$, as shown in Fig. 5. This strongly suggests that the partitioning is caused by the purely geometric reason.

Another interesting feature is the way of coalescence between compartments. It is found that coalescence occurs only between the compartments having the same parity. Two compartments having a different parity never coalesce even if they touch with each other. Thus, the parity is preserved during coalescence and particles are

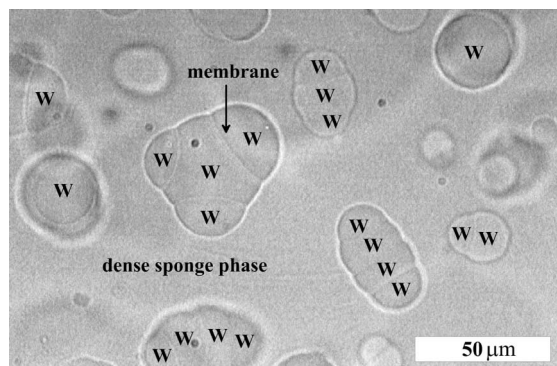


FIG. 3. Pattern observed under contrast-enhanced optical microscopy in the coexistence region of a dilute micellar (water, denoted by W) and a dense sponge phase (denoted by DS) in the absence of particles. A system is an aqueous solution of $C_{12}E_5$ (2.5 wt % $C_{12}E_5$). The temperature was 72.0°C .

kept completely partitioned during the coarsening process of cellular droplets. Since the parity is preserved during coarsening, a system eventually phase separates into three macroscopic phases: the water phase with particles, that without particles, and the dense sponge phase without particles.

It should be mentioned that we confirm the same phenomenon is also observed for any combinations of membranes ($C_{12}E_5$ and $C_{10}E_3$) and balls (polystyrene latex and silica particles). A typical example for a combination of $C_{12}E_5$ and silica particles ($d = 80$ nm) is shown in Fig. 2(b). Thus, the phenomena are not specific to particular combinations of membranes and balls. This and the above geometric criterion for the occurrence of the spontaneous partitioning of particles suggest the universal nature of this phenomenon.

From detailed observation of a system without particles under contrast-enhanced microscopy, we find that a domain of a water phase coexisting with the dense sponge phase is always composed of compartments separated probably by a single bilayer membrane. The compartment has a parity of inside (I) and outside (O) due to the underlying symmetric-asymmetric phase transformation upon phase separation, although the I and O regions are chemically identical. This cell structure should be common to both membrane systems with and without balls [compare Figs. 2(a) and 3]. We can say that by putting particles, a hidden symmetry-breaking transformation becomes evident and visible. Here we propose a model for a cell structure of water domains separated by membrane walls, which can naturally explain why coalescence only between water compartments having the same parity (parity-preserving coalescence) is allowed. The possible membrane organization above T_{PS} is schematically shown in Fig. 4. With this cell structure, we can explain the parity conservation of domains during coarsening. The reconnection of membranes at the boundary of colliding domains can happen only for a case that there

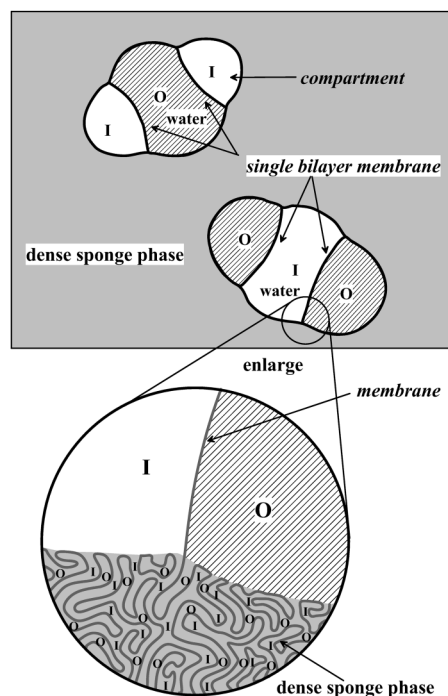


FIG. 4. A schematic phase structure in the coexistence region of a sponge and a dilute micellar (or water) phase. When a sample is heated above T_{PS} , a water phase emerges from a sponge phase and grows with time. The details of the membrane organization is schematically shown in the inset. We can assign all the domains in a dense sponge phase to the inside (I) and outside (O) ones. The alternative nature of I and O should be strictly preserved. This feature comes from the fact that membranes are never disconnected and broken up in a usual situation.

exist an even number of membranes between them. The reconnection of an odd number of membranes is difficult since it must accompany breakup of membranes itself, which costs energy far beyond the thermal one. Upon collision of domains with different parities, thus a single membrane eventually remains between two domains. On the other hand, water domains with the same parity are directly connected with each other by water channels in the sponge phase. Thus, they can coalesce. The parity is preserved in this way during coarsening.

Finally we consider how particles are spontaneously partitioned upon phase transformation from a homogeneous sponge phase to coexisting dense sponge and water phases. This phenomenon requires an underlying symmetry-breaking transition, which gives the parity to chemically identical water compartments (see Figs. 3 and 4). As a candidate of such a transition, the symmetric-asymmetric ($S-A$) sponge phase transition [9] comes into mind. Our static and dynamic light scattering, rheology, and differential scanning calorimetry studies, however, suggest the absence of such a symmetry-breaking transition inside the one-phase region of the sponge phase (the details will be described elsewhere). With this in

mind, thus a plausible scenario is that our case corresponds to the extreme case of the *S-A* sponge transition [9]; namely, the phase separation of a sponge phase into a dense sponge and a dilute micellar phase should be regarded as the strongly first-order *S-A* sponge transition. Note that in this scenario the dense sponge phase corresponds to the symmetric sponge, while a dilute micellar phase corresponds to the extreme situation of the asymmetric sponge.

According to this picture, the partitioning of particles can be explained as follows. In the initial stage of phase separation during or after a temperature jump, the homogeneous symmetric sponge, which is formed when we keep a system in the one-phase sponge region, is transformed into the asymmetric one. The asymmetric sponge is divided by a bilayer membrane into a region having smaller volume inside (*I*) and that having a larger one outside (*O*). The characteristic size of *I* domains quickly approaches to the characteristic size of the final dense sponge phase. In this process, the particles are expelled from *I* domains and go into *O* ones, if the characteristic size of *I* domains becomes smaller than the particle diameter *d* and the process is slow enough [10]. This may be the first step of partitioning. Thus, particles exist only in the *O* domains after this process. Note that this scenario is consistent with the geometrical criterion for the occurrence of the phenomenon (see Fig. 5).

The next important step is the process of macroscopic partitioning of particles into cellular structures upon the phase separation of a transiently formed asymmetric sponge into a dense symmetric sponge and a water phase. This strongly first-order *S-A* transition can occur by nucleating droplets of pure water in the sponge phase. This can happen in two ways: by nucleation of an *I* water droplet or by nucleation of an *O* water droplet [11]. Note that particles exist only in the *O* domains after the first step. Thus the *O* droplets contain particles, while the *I* droplets do not. We stress that domains *I* and *O* coalesce only with domains of the same type and grow in their size while keeping the parity. This is due to the alternating nature of inside and outside regions, which are separated by single membranes. The coarsening process should be initiated by the direct contact between membranes and followed by the reconnection of the membranes to produce the topological change. During this process, particles cannot come back to the inside region since coalescence can occur only between the domains of the same type. This feature supports a picture that the free energy cost of edges or seams in the bilayer is so large that such defects are to be absent [9]. We now believe that the above-described scenario is physically plausible. If this is the case, the phenomenon found here is a direct confirmation of the classical picture of symmetry breaking in the sponge phase and the continuity of bilayer membranes [9]. Since the discussion is speculative, however, further

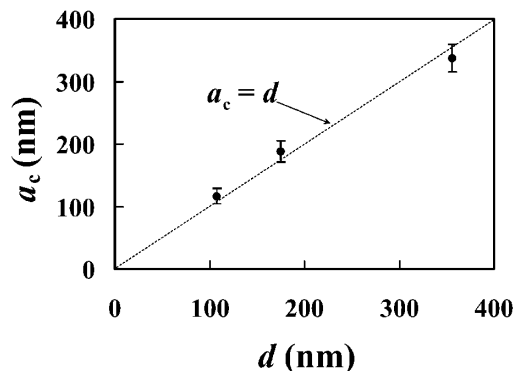


FIG. 5. The threshold spacing of the sponge phase a_c , which is obtained from $a_c = \alpha\delta/\phi_c$, is plotted against the diameter of latex particles d . This clearly indicates that $a_c \sim d$.

studies are highly desirable to elucidate the detailed physical mechanism of this unusual phenomenon.

The phenomenon found here provides us with a new physical principle for the intentional partitioning of nanomaterial, which is unique in the sense that it applies the topological transformation of a membrane system to material partitioning. It should be stressed that the purely geometrical nature of the criterion for the occurrence of the phenomenon suggests its universal nature.

The authors thank M. E. Cates for his invaluable comments on the mechanism. One of the authors (H. T.) is also grateful to T. C. Lubensky and G. Porte for fruitful discussion. This work was partly supported by a grant-in-aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

*To whom correspondence should be addressed.

[†]Present address: Yokoyama nano-structured liquid crystal project, Tsukuba 300-2635, Japan.

- [1] See, e.g., *Soft Matter Physics*, edited by M. Daoud and C. E. Williams (Springer, Berlin, 1999).
- [2] W. Helfrich, *Z. Naturforsch.* **33a**, 305 (1978).
- [3] A. Ott *et al.*, *Europhys. Lett.* **12**, 395 (1990).
- [4] C. Ligoure *et al.*, *Phys. Rev. Lett.* **71**, 3600 (1993).
- [5] J. O. Radler *et al.*, *Science* **275**, 810 (1997).
- [6] G. Salamat and E. W. Kaler, *Langmuir* **15**, 5414 (1999).
- [7] R. Strey *et al.*, *J. Chem. Soc. Faraday Trans.* **86**, 2253 (1990).
- [8] G. Porte *et al.*, *J. Phys. (Paris)* **50**, 1335 (1989).
- [9] D. Roux *et al.*, *J. Phys. Chem.* **96**, 4174 (1992).
- [10] We note that the temperature may also be high enough to allow particles to cross the membrane.
- [11] We note that the nucleation rate of water pockets during the strongly first-order *S-A* transition is almost the same for *I* and *O*. This may be because the large change in the concentration in the sponge phase during the transition smears out the slight asymmetry in the asymmetric sponge that is formed in the first step. This point needs further study to be clarified.