

## Fractal Growth of Crystalline Phospholipid Domains in Monomolecular Layers

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Self-similar structures of single-crystalline domains in lipid monolayers at the air-water interface are observed after a stepwise increase of the surface pressure. It is shown that these fractal structures with Hausdorff dimensions of  $d \approx 1.5$  anneal because of the influence of edge tension on time scales of minutes. The results can be understood within the framework of diffusion-limited aggregation taking into account constitutional supercooling due to different miscibilities for impurities in solid and in fluid lipid phases.

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Self-similar structures are observed in many physical systems as well as in biology.<sup>1</sup> They often result from crystallization or aggregation under highly nonequilibrium conditions.<sup>2</sup> As a candidate for these structures to form within cell membranes the crystallization of phospholipids may be envisaged. For monolayers at the air-water interface, single-crystalline lipid domains can be investigated by fluorescence microscopy.<sup>3-5</sup>

The film balance with integrated fluorescence microscope<sup>6</sup> provides the following features essential for this study: (1) As a result of observation with a SIT television camera and tape recording the time resolution is as high as forty recorded images per second. Yet there is a dead time of about 2 sec due to surface waves which follow a pressure jump and move the monolayer out of focus. (2) Its high sensitivity enables observation of monolayers containing the impurity dye in concentrations as low as 0.1 mol%. (3) Without application of convection barriers that might limit the fast pressure equilibration, surface flow could be kept below 5  $\mu\text{m}/\text{sec}$ . Thus changes in domains could be followed over time periods of minutes. Solid domains appear dark in the fluorescence micrographs as a result of negligible dye solubility in the solid phase.

The phospholipid used, *L*- $\alpha$ -dimyristoylphosphatidylethanolamine (DMPE) (Fluka, chromatographically pure), is distinguished by an extremely flat surface-pressure-to-area diagram in the region of coexistence between fluid and solid phases, as observed in Fig. 1 for three different dye concentrations. The distinguished break in the slope of the diagram at the pressure  $p_c$  corresponds to the onset of the fluid-to-solid phase transition. Obviously  $p_c$ , which may be called melting pressure, increases with dye content. A slight increase of the pressure to a value above  $p_c$  strongly increases the degree of crystallization. The dyes incorporated, meta-tetra-phenyl-(*bis*-2-oxybenenic acid)-porphyrin (TPheP) and tetra-3-eicosylpyridinium porphyrin bromide (TPyP), a generous gift of Dr. A. Ruaudel-Teixier, Paris, revealed identical surface textures. TPyP was used predominantly because it provides the additional advantage of negligible self-quenching. Thus fluorescence intensity depends linearly on dye concentration and local concentrations

can be measured via fluorescence intensity distribution.

The water used was Millipore filtered. The subphase was adjusted to a *pH* of 7 via 25-mM phosphate buffer;  $T = 20^\circ\text{C}$ . Rapid pressure increases (jumps) were effected by moving a barrier to change the mean molecular area at a speed of up to  $10^{-2} \text{ nm}^2/\text{sec}$ .

For an intermediate dye concentration ( $\sim 0.7$  mol%) it is possible to follow the formation and growth of dendrites (Fig. 2) resembling those calculated for diffusion-limited aggregation. These dendrites disappear and circular domains form if we maintain a constant area over time periods of 5–10 min.

Variation of dye content in this study basically served two purposes: (i) The dye acts as a typical surface-active impurity, the influence of which can therefore be studied. (ii) With selection of a proper dye concentration the time scale of crystallization can be varied and thus different stages of crystal growth become observable.

Concerning (i) we will show constitutional supercooling governing dendrite formation. Domain growth strongly depends on dye concentration and heat conduction into the subphase is probably efficient enough to prevent heat accumulation between dendrite tips.

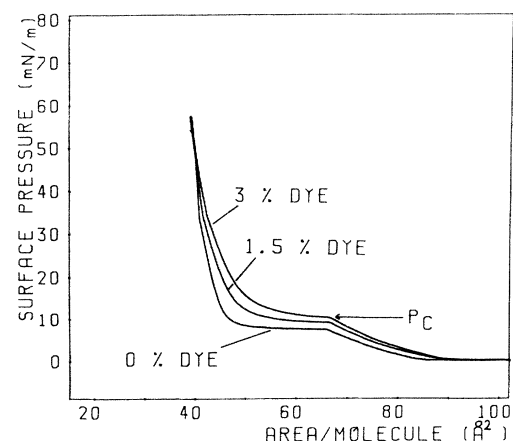


FIG. 1. Surface pressure as a function of molecular area for a DMPE monolayer containing increasing concentrations of the dye TPyP ( $T = 20^\circ\text{C}$ , *pH* 7).

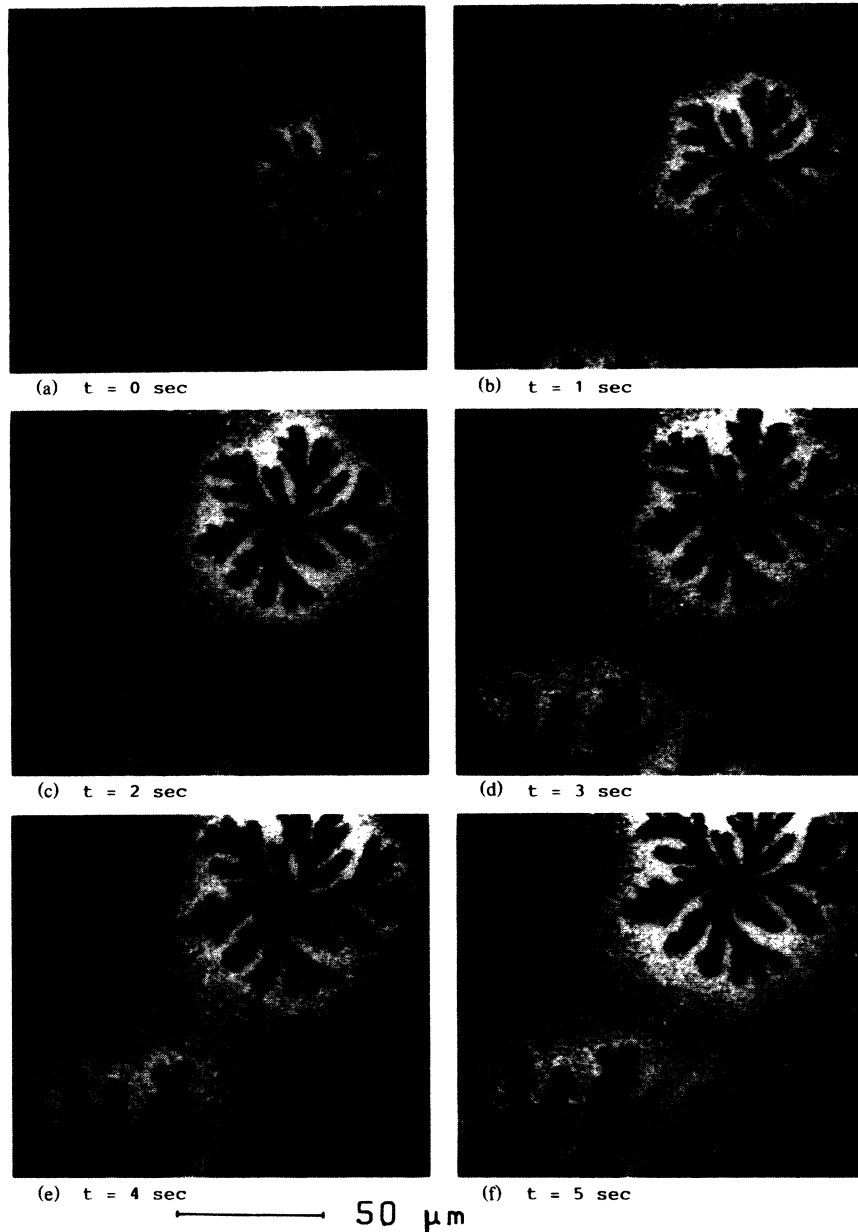


FIG. 2. Time development of solid DMPE domains following a fast compression ( $\approx 10^{-2} \text{ nm}^2/\text{molecule} \cdot \text{sec}$ ) to increase the surface pressure from below to about  $0.5 \text{ mN/m}$  above the transition pressure.  $t=0$  marks the time where the solid domain could first be observed. Concentration of TPyP,  $0.7 \text{ mol\%}$ . Fluid areas near the solid domains appear very bright because of dye enrichment near the solid-fluid interface. Areas further removed in the fluid phase appear homogeneously intense, as can better be quantified densitometrically.

The dye TPyP which causes an increase in the pressure  $p_c$  corresponding to the phase transition, which was measured by us to be  $0.8 \text{ mN/m}$  per  $\text{mol\%}$  dye, is squeezed out of the solid phase and accumulates at the phase boundary. This can be visualized and quantified in a densitogram (Fig. 3). In the simplest one-dimensional case the concentration  $c(x)$  as a function of distance  $x$  from the phase boundary is given by<sup>7</sup>

$$c(x) = c_{00} \left[ 1 + \frac{1-k}{k} \exp\left(-\frac{vx}{D}\right) \right]. \quad (1)$$

In Eq. (1)  $k$  is the partition coefficient for the dye between fluid and solid phase. The growth velocity  $v$  can be measured in an experiment like that of Fig. 2.  $c(x)/c_{00}$ ,  $c_{00}$  being the dye concentration far away from the boundary, is deduced from the densitogram in Fig. 3. From this the diffusion coefficient  $D$  can be calculated. One obtains a value of  $2 \times 10^{-7} \text{ cm}^2/\text{sec}$ , which is within the range of values reported for phospholipid monolayers in the fluid phase.<sup>4</sup> This again supports the idea that the crystallization process is con-

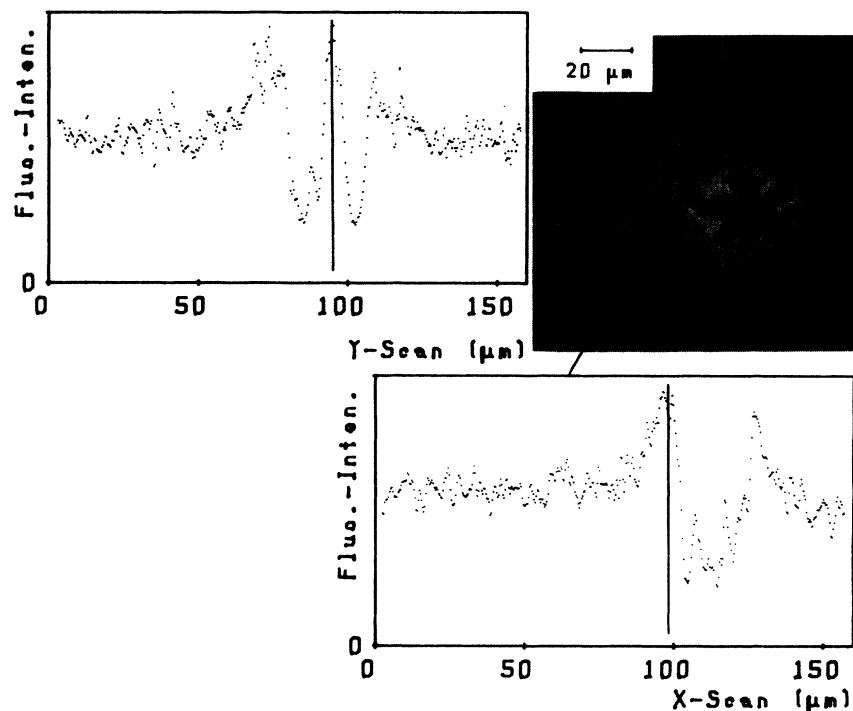


FIG. 3. Fluorescence intensity along two orthogonal directions indicated by the bars in the picture. The densitograms were obtained by transferring the video signal to the image analysis system, smoothing, and correcting for inhomogeneous illumination.

trolled by constitutional supercooling.

From the experiment in Fig. 3 one can also estimate the increase in melting pressure due to impurities concentrating at the interface and compare this with the deviation  $\Delta p_{\text{expt}}$  of the pressure from the equilibrium value shortly after the jump ( $\Delta p_{\text{expt}} = 0.5 \pm 0.1$  mN/m). One can assume  $c_{00} = 0.7$  mol%, corresponding to the relative weight, as only a small fraction of the monolayer is solidified. From the determined value  $c(x)/c_{00} \approx 2.5$  one then determines a dye-concentration increase of 1 mol% at the interface, corresponding to a melting-pressure increase of  $\Delta p(x=0) = 0.8$  mN/m. The fact that this value is larger than  $\Delta p_{\text{expt}}$  explains why crystallization is not possible in the areas of highest dye content, i.e., between the dendrite arms. On the other hand, it suggests the existence of local equilibrium at the dendrite tips where the dye concentration is smaller. Thus crystallization is limited by diffusion of the dye from the interface. We may therefore use concepts that consider diffusion-limited aggregation and analyze our dendrites with respect to fractal symmetry.

Clearly dendrites with smooth boundary lines forming at longer times after crystallization are not fractal structures. In this case edge tension is obviously effective in reducing the length of the solid-liquid boundary. Therefore the most promising candidates displaying fractal behavior are those observed at low dye concentration. These were therefore analyzed and tested for the following scaling behavior:

(1) The circumference  $U$  measured as described by Nittman, Daccord, and Stanley<sup>8</sup> with rulers of length  $nl_0$  ( $n = \text{integer}$ ,  $l_0 = \text{unit length}$ ) is given by

$$U(n) = \text{const} \times n^{-d} \times (nl_0). \quad (2)$$

(2) The fractal dimension should also appear in a measurement of the fraction of solid area  $F(R)$  within a square of edge length  $2R$ .<sup>1,2</sup> One expects

$$F(R) = \text{const} \times R^d. \quad (3)$$

A logarithmic plot of  $U$  in units  $N$  of ruler lengths  $nl_0$  vs  $n$  shows a power-law dependence for intermediate lengths corresponding to values between 3 and 25  $\mu\text{m}$  [Fig. 4(a)]. For this range an exponent of  $d = 1.5 \pm 0.1$  can be determined. For shorter dimensions deviations from this power law are encountered as a result of limited experimental resolution (2  $\mu\text{m}$ ) and, above all, as a result of edge tension reducing sharp edges. Edge tension is also responsible for the fact that these highly carved domain boundaries are observed only for short times after formation. Approaching the other extreme in dimensions, fractal growth is of course limited by domain size. The same arguments hold if we analyze the area according to Eq. (3) [Fig. 4(b)]. There one realizes that for intermediate dimensions the power law with  $d = 1.5$  is reasonably well fulfilled. We additionally realize that the fractal dimension  $d = 1.5$  derived in the analysis is considerably smaller than that calculated for diffusion-limited aggregation ( $d = 1.67$ ).<sup>2</sup> This was

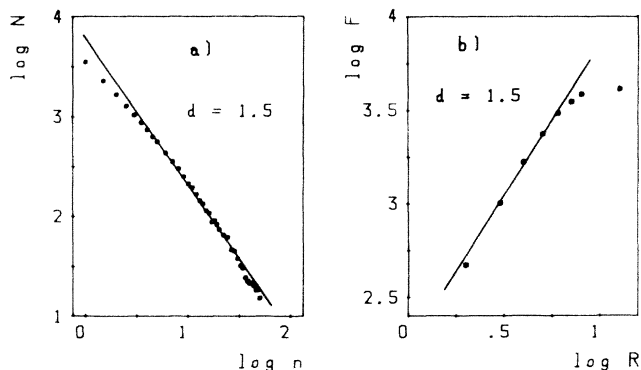


FIG. 4. (a) Circumference in units of  $nl_0$ , i.e.,  $N = U(n)/nl_0$  ( $l_0 = 0.8 \mu\text{m}$ ), as a function of  $n$  and (b) solid area  $F$  within a square of edge length  $2R$ .  $R = 1$  corresponds to  $8 \mu\text{m}$ . The boundary lines of a dendrite were read into a personal computer via a graphics table and then analyzed according to Eqs. (2) and (3).

also observed by Nittman, Daccord, and Stanley<sup>8</sup> and ascribed to the finite dimension of their Hele-Shaw cell. Similar arguments may also apply in the present case where the finite dimension is determined by a neighboring crystallite competing for monomer aggregation. There is, however, also another force that may be responsible for the smaller fractal dimension: Long-range electrostatic forces were shown to exist between the polar molecules at the interface.<sup>9</sup> These repulsive forces are difficult to include in a calculation but may enforce formation of elongated structures. Whereas this force definitely exists, we cannot exclude, but have no indication for, convective transport which may also be responsible for the reduced fractal dimension.

To assess the degree of supercooling where these fractal structures were observed we can use Eq. (2.6) of Langer and Müller-Krumbhaar<sup>10</sup> which in the present case reads

$$\Delta = [c(x) - c_{00}]/c_{00}. \quad (4)$$

Thus  $\Delta$  can be estimated from the densitograms at various dye contents to be between 0.1 and 1.5, where the upper value corresponds to the condition of fractal growth.

Finally we should mention that although the dye does not tend to condense and thus form a nucleation center as was observed for other porphyrins,<sup>11</sup> its pres-

ence increases the density of solid domains. This points to the possibility of a reduced edge tension by an increase of the dye concentration. Thus, the critical size for a nucleus to grow is reduced and the number of growing nuclei is increased. The reduced edge tension may also be the reason why we could make a large pressure jump ( $0.5 \text{ mN/m}$ ) at low dye content but only a very small one at high dye concentrations. In the latter case, nuclei already become critical at very low supersaturations.

We are aware that this is a study with a highly artificial system, yet we would like to point out that the process considered may be responsible for the formation of fractal structures on cell surfaces. In the latter case structures may be formed mostly by chemical changes, but under highly nonequilibrium conditions, thus exhibiting the high boundary-to-area ratio which is favorable for interfacial reactions.

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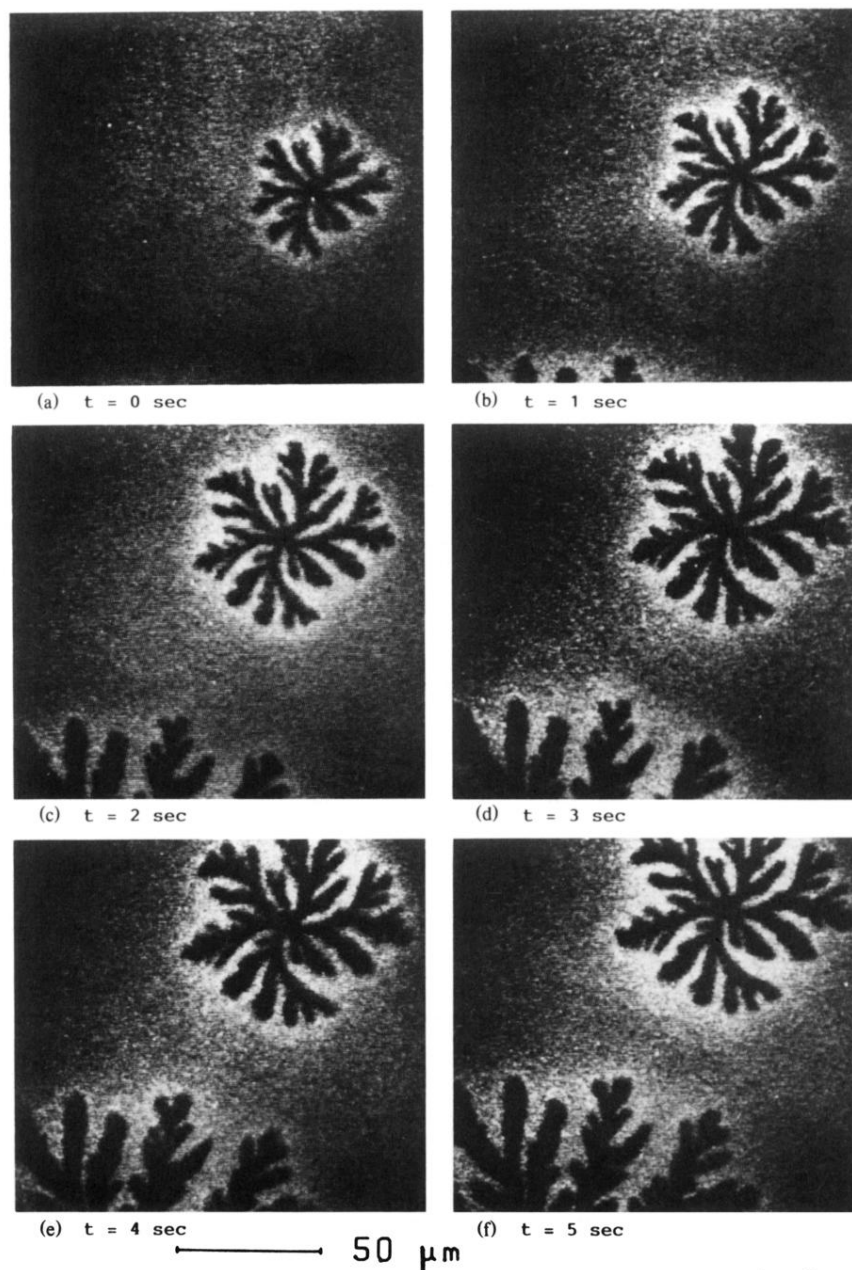


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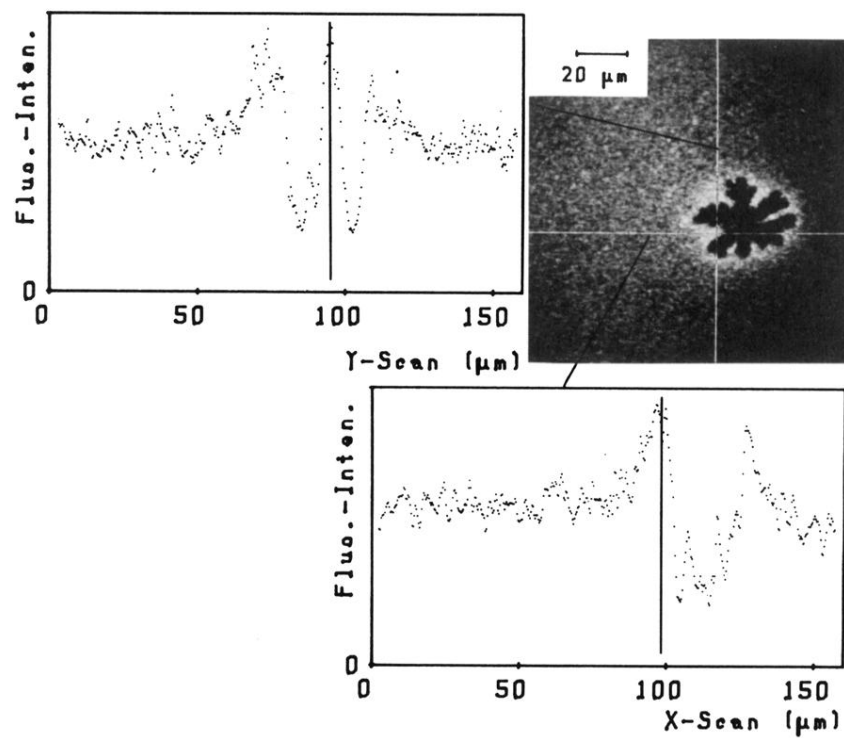


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