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**Statistical Mechanical Determination of nanocluster size distributions
in the phase coexistence region of a first order phase transition from
the isotherms of DMPC monolayers at the air-water interface**

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Abstract

A statistical mechanical deconvolution procedure for the experimentally measured surface pressure – area isotherms has been presented to obtain the surface pressure dependence of the liquid expanded (LE) and liquid condensed (LC) nanocluster size distributions in the LE – LC phase coexistence region of the first order phase transition of Dimyristoyl phosphatidylcholine (DMPC) monolayers at the air-water interface. This study presents the application of the deconvolution formulation introduced originally by Freire and Biltonen for experimentally measured specific heat to calculate submicroscopic lipid cluster distribution function in the phase coexistence region [E. Freire, R. L. Biltonen, Biopolymers, 1978, 17, 481-496] and carries their formulation to surface pressure isotherms. The present procedure involves the extraction of the pressure partition function calculated from the isotherm and utilizes the general relation between molecular density fluctuations and macroscopic lateral compressibility. In this procedure the high-density LC phase boundary has been determined *uniquely*. The average nanoscopic cluster sizes obtained in this study have been compared with the results from previous experimental studies. The cause for the finite difference between the values of LC phase boundary area obtained from each of the present deconvolution procedure and the conventional extrapolation method to the same isotherm has been discussed from the viewpoint of slow hierarchical growth from nanoscopic clusters to macroscopic domains in the coexistence region.

Introduction

The determination of the surface pressure (π) – molecular area (A) isotherms is the most common macroscopic measurement that is performed on monolayers formed at the interface between air and water (Langmuir monolayers).¹ Much of our knowledge of the phase behavior of monolayers has been gained from such studies.² Over several past decades fluorescence microscopy (FM)^{3,4} and Brewster angle microscopy (BAM)^{5,6} have allowed the inferred phases and the transitions between them of these monolayers to be determined by direct observation.^{7,8} Of the isotherms that exhibit transitions, the isotherm behavior in the liquid expanded (LE) – liquid condensed (LC) phase transition region has been much addressed due to the appearance of a non-horizontal plateau in the coexistence region.^{9,10,11} Although it was suggested that this transition is not first-order, FM and BAM clearly showed the two-phase coexistence (or a heterogeneous pattern composed of the LE and LC domains) through the transition region and the changes in the relative amounts of the phases followed the lever rule.⁷ The first-order character of this broad transition is thus no doubt. The results of the computer simulation study of cooperative phenomena in lipid monolayers and bilayers showed a first-order transition with strong lateral density fluctuations.¹² The density fluctuations in monolayers manifested themselves *microscopically* as formation of dynamic clusters within the thermodynamic majority phase theoretically.¹³ Experimental observations by transmission electron microscopy (TEM)^{14,15} and atomic force microscopy (AFM)^{16,17,18} revealed the existence of nanoclusters in the LE-LC coexistence region of monolayers transferred onto *solid* substrates. Direct observations by optical microscopy of such nanoscopic clusters on the *water surface* is, however, hampered by the fact that

the length scale of the clusters is in the nanometer range that is beyond the resolution of optical microscopy. Angelova et al. made experimental observations of three-dimensional cluster nucleation and growth of domains in supersaturated monolayers.^{19,20,21} In relation to the non-horizontal coexistence plateau in the isotherm Albrecht et al. attributed a finite slope of the isotherm to the limited cooperativity of the transition and estimated the size of the cooperative clusters using the van't Hoff approach combined with the relation between the order parameter and the equilibrium constant.²² For the π dependence of cooperative cluster size during the transition the above study was extended to the use of the experimental integral equation that involved isotherm and compressibility data.²³ Freire and Biltonen demonstrated that the partition function of a bilayer system is experimentally accessible by means of the integral equation from scanning calorimetric data of lipid bilayers.²⁴ They applied a statistical mechanical deconvolution procedure based on the canonical approach to the measured specific heat to produce a lipid domain (submicroscopic cluster) distribution function in the gel-liquid crystalline phase coexistence region of the temperature-driven main transition that was consistent with the measured specific heat. In their study the experimental partition function describing the gel or the liquid-crystalline cluster distribution used the single gel or liquid-crystalline state as a reference state and was defined by the definite integral in which depending on the choice of the reference state the onset temperature or the end temperature was included. Here, it should be noted that the determination of both LE (liquid-crystalline) and LC (gel) phase boundaries is required for the estimation of the cooperative cluster size during the isothermal or isobaric transition in all the above methods.^{22,23,24}

Concerning the phase boundaries in the LE-LC transition the measurement of the molecular area A_{LE} of the low density LE phase boundary can be made normally rather accurately from a break in the slope of the isotherm. We have, however, difficulty in accurately determining the high density LC phase boundary A_{LC} , since the termination of the phase transition on compression is not clearly observed in the isotherm.⁹ This boundary has often been estimated as the intersection of the LE-LC plateau and the extrapolation of the steep portion of the isotherm beyond the transition from the isotherm.^{25,26,27} There has been, however, so far no definite method of determining the value of A_{LC} without ambiguity from only a single isotherm measurement. To detect the boundaries of the transition the area fraction of LC phase domains as a function of molecular area has been compared with the isotherm by using optical microscopy with image analysis technique. The combined study of BAM with the lever rule technique using image analysis showed that the molecular areas extrapolated to the coexistence boundaries with the lever rule correspond well to those estimated by the tangential extrapolation method to the compression isotherm.²⁸ There have, on the other hand, often been reported apparent violations of the lever-rule, with *much less* LE or LC molecular area observed than that expected from isotherm result.^{29,30,31,32} Here it may be important to remember that the fluctuation - dissipation (FD) theorem in equilibrium provides relationships between thermodynamic response functions and variances in the corresponding physical quantities.³³ The microscopic cooperative clusters are measures of lateral density fluctuations that can be closely related to the isothermal compressibility as a thermodynamic response function macroscopically via the FD theorem. To obtain

some information about both the nanoscopic cluster growth and the relations between nanoscopic clusters and macroscopic domains in the two-phase coexistence region, in this study we develop a statistical mechanical deconvolution scheme to experimental isotherm data that allows the *simultaneous* determination of LE and LC nanoscopic cluster size distribution functions and LC phase boundary in the first order LE-LC phase transition.

Experimental

Dimyristoyl phosphatidylcholine (DMPC) was purchased from Avanti Polar Lipids, Inc. and claimed to be >99% pure. Lipids were dissolved in *n*-hexane: ethanol (9:1) to a concentration between 0.4 and 0.5 mmol/l, and used without further purification. The solution was spread onto the pure water (Millipore Milli-Q at 18 Ω cm, pH 5.5) contained in a custom-built Teflon trough. After evaporation of the solvent for 20 – 30 min, the monolayer was compressed at a rate of 0.01 nm²/molecule/min. Langmuir isotherms were obtained by compressing monolayers and the monolayer surface pressure (π) and the molecular area (A) were automatically stored on disk. The isothermal area compressibility κ_T^A was calculated from the digitally stored isotherm data. Thermocouples immersed in the subphase were used to measure the subphase temperature and the subphase temperature was controlled within 0.1 °C by circulating water and two water cooled Peltier elements mounted directly on the back side of the copper plate. The copper bottom was coated with a 0.3 mm thin Teflon foil. The surface pressure - area isotherms were measured using a commercial film balance (R&K GmbH, Potsdam, Germany) equipped with Wilhelmy-type pressure measuring system. The surface pressure sensibility was 0.1

mN/m.

Theoretical Background

Freire and Biltonen developed a statistical mechanical deconvolution process based on the canonical and grand canonical approach^{24,37} to the measured specific heat to produce a submicroscopic lipid cluster distribution function in the phase coexistence region of the temperature-driven main transition. In analogy with their approach, we have formulated a deconvolution scheme to the isotherm utilizing the general relation between the pressure partition function and the generalized partition function³⁴ to produce LE and LC cluster size distribution functions in the phase coexistence region of the isothermal first order LE - LC transition. The present deconvolution procedure also utilizes the equilibrium FD relation from which the crossover-point of LE and LC cluster distribution curves coincides with the peak position of isothermal compressibility at the transition midpoint in the diffuse LE-LC transition.^{35,36,37}

The pressure partition function $\mathcal{Y}$, the generalized partition function Ψ , and the cluster partition function Ω

We consider a monolayer in the two phase coexistence region of the first order LE-LC transition. Let $m^{LE}(N^{LE})$ and $m^{LC}(N^{LC})$ be the total number of clusters (molecules) in LE and LC phases, respectively, and $e^{-\pi m_k^i k a / k_B T}$ the statistical weight of i ($\in$ LE or LC) phase cluster containing k molecules (m_k^i : the number of i phase clusters containing k molecules, a : the average area per

molecule). For the cluster model^{24,37}, assuming that individual clusters can be regarded as independent subsystems (no interactions exist between them), the separation into a product between LE and LC clusters is possible and the surface pressure partition function $Y(m^{LE}, N^{LE}; m^{LC}, N^{LC})$ in the monolayer system can be written as follows:

$$Y(m^{LE}, N^{LE}; m^{LC}, N^{LC}) = Y^{LE}(m^{LE}, N^{LE}) \cdot Y^{LC}(m^{LC}, N^{LC})$$

m^i is the total number of i phase clusters ($= \sum_{k=1}^{\infty} m_k^i$), N^i is the total number of i phase molecules ($= \sum_{k=1}^{\infty} km_k^i$). The pressure partition function, $Y^i(N^i, T, \pi)$ of i phase can be written as follows:

$$Y^i(N^i, T, \pi) = \sum_{\{m^i\}} W(m_k^i, N^i) e^{-\pi \sum_{k=1}^{\infty} m_k^i ka / k_B T} = \sum_{\{m^i\}} \prod_{k=1}^{\infty} \frac{N^i!}{m_k^i!} e^{-\pi \sum_{k=1}^{\infty} m_k^i ka / k_B T},$$

where the summation runs over all sets of numbers of i phase clusters $\{m^i\}$. The degeneracy factor W is the number of different ways of distributing N^i indistinguishable molecules into m_k^i boxes without restricting the occupation number per box. The partition function $\Psi^i(\alpha, T, \pi)$ of the generalized ensemble can be expressed by a polynomial expansion in the activity term $e^{-\alpha}$ and the coefficients of this expansion are the pressure partition functions related to the Gibbs free energy of the system. The generalized partition function $\Psi^i(\alpha, T, \pi)$ for the cluster model can be expressed as a product of cluster grand partition functions Ω_{π}^i finally:

$$\begin{aligned} \Psi^i(\alpha, T, \pi) &= \sum_{N^i} Y^i(N^i, T, \pi) e^{-\alpha N^i} = \sum_{\{m^i\}} \prod_{k=1}^{\infty} \frac{N^i! e^{-\pi \sum_{k=1}^{\infty} m_k^i ka / k_B T}}{m_k^i!} e^{-\alpha \sum_{k=1}^{\infty} km_k^i} \\ &= \left(\sum_{k=1}^{\infty} e^{-\pi ka / k_B T} e^{-k\alpha} \right)^{m^i} = (\Omega_{\pi}^i)^{m^i}. \end{aligned}$$

We used the multinomial theorem to go from the first line to the second line. The whole partition function for the cluster model, $\Psi(\alpha, T, \pi)$ can also be written formally in terms of cluster partition function, Ω_π^i as $\Psi(\alpha, T, \pi) = \sum_{\{m^i\}} \prod_{i \in \text{LE or LC}} (\Omega_\pi^i)^{m^i}$, where the summation runs over all sets of number of clusters $\{m^i\}$ consistent with the specification of the system. It is useful to define reference states to which to refer the relative statistical weights³⁷ and we choose the LE state as reference state. Taking the partition function giving LE(LC) state a statistical weight equal to one ($Y^{\text{LE(LC)}} = \exp\left(-\left(g - g^{\text{LE(LC)}}\right)/k_B T\right)$, g is the Gibbs free energy per molecule), the average cluster size distribution function $k^i(\pi)$ and the relative probability of the cluster size $P^i(k)$ of i phase can be calculated from the experimental surface pressure partition functions, $Y^i(N)$ using the relation between the pressure partition function and the cluster grand partition function. $Y^i(N)$ can be represented from the relation between the surface area and the pressure partition function, $a = -k_B T (\partial \ln Y / \partial \pi)_{N, T}$, as follows:

$$Y^{\text{LE}}(N) = \exp\left[\frac{1}{k_B T} \int_{\pi_{\text{LE}}}^{\pi} (a_{\text{LE}} - a) d\pi\right] \quad \text{and} \quad Y^{\text{LC}}(N) = \exp\left[\frac{1}{k_B T} \int_{\pi}^{\pi_{\text{LC}}} (a - a_{\text{LC}}) d\pi\right], \quad (1)$$

where $a_{\text{LE}}(a_{\text{LC}})$ and $\pi_{\text{LE}}(\pi_{\text{LC}})$ are the area and the surface pressure at which all molecules exist in the liquid expanded (condensed) phase, respectively. Defining the ratio $s = Y^{\text{LE}}(N)/Y^{\text{LC}}(N)$, the statistical weight of a cluster containing k molecules in the LC phase is given by s^k .

The LE and the LC cluster partition functions are given by the equations using the above experimental partition functions, $Y^{\text{LE}}(N)$ and $Y^{\text{LC}}(N)$:

$$\Omega_{\pi}^{\text{LE}} = \sum_{k=1}^{\infty} e^{-k\alpha} \quad \text{and} \quad \Omega_{\pi}^{\text{LC}} = \sum_{k=1}^{\infty} k \left(\frac{Y^{\text{LE}}}{Y^{\text{LC}}} \right)^k e^{-k\alpha},$$

where the LE phase has been chosen as reference state. For very large systems ($N \rightarrow \infty$), $Y^{\text{LE}}(N)$ and $Y^{\text{LC}}(N)$ approach a limit Y^{LE} and Y^{LC} , which are independent of N (thermodynamic limit). In the thermodynamic limit ($e^{\alpha} \rightarrow e^{\alpha_0} = Y^{\text{LE}}$), the average LC phase cluster size $k^{\text{LC}}(\pi)$ is given by the equation:

$$\begin{aligned} k^{\text{LC}}(\pi) &= - \left(\frac{\partial \ln \Omega_{\pi}^{\text{LC}}}{\partial \alpha} \right)_{\alpha=\alpha_0} \\ &= \frac{\sum_{k=1}^{\infty} k \left(e^{-\pi a / k_B T} \right)^k e^{-k\alpha_0}}{\sum_{k=1}^{\infty} \left(e^{-\pi a / k_B T} \right)^k e^{-k\alpha_0}} = \frac{\sum_{k=1}^{\infty} k s^k e^{-k\alpha_0}}{\sum_{k=1}^{\infty} s^k e^{-k\alpha_0}} = \frac{\sum_{k=1}^{\infty} k \left(\frac{Y^{\text{LE}}}{Y^{\text{LC}}} \right)^k Y^{\text{LE}-k}}{\sum_{k=1}^{\infty} \left(\frac{Y^{\text{LE}}}{Y^{\text{LC}}} \right)^k Y^{\text{LE}-k}} = \frac{\sum_{k=1}^{\infty} k (Y^{\text{LC}})^{-k}}{\sum_{k=1}^{\infty} (Y^{\text{LC}})^{-k}} = \frac{Y^{\text{LC}}}{Y^{\text{LC}} - 1}. \quad (2) \end{aligned}$$

In an analogous procedure, we can obtain $k^{\text{LE}}(\pi) = Y^{\text{LE}} / (Y^{\text{LE}} - 1)$. Using these relations we can obtain $k^i(\pi)$ ($i \in \text{LE}, \text{LC}$) from the measured isotherm.

Equilibrium Fluctuations of the Cluster Size

The probability density, $p^{\text{LC}}(k)$, of finding an LC cluster of k molecules among all LC clusters is²⁴:

$$p^{\text{LC}}(k) = \frac{s^k e^{-\alpha_0 k}}{\Omega_{\pi}^{\text{LC}}} = \frac{(Y^{\text{LC}})^{-k}}{\sum_{k=1}^{\infty} (Y^{\text{LC}})^{-k}} = \frac{Y^{\text{LC}} - 1}{(Y^{\text{LC}})^k}, \quad \left(\sum_{k=1}^{\infty} p^{\text{LC}}(k) = 1 \right).$$

In an analogous procedure, the probability density, $p^{\text{LE}}(k)$, of finding an LE cluster of k molecules among all LE clusters is:

$$p^{\text{LE}}(k) = \frac{1^k e^{-\alpha_0 k}}{\Omega_{\pi}^{\text{LE}}} = \frac{(Y^{\text{LE}})^{-k}}{\sum_{k=1}^{\infty} (Y^{\text{LE}})^{-k}} = \frac{Y^{\text{LE}} - 1}{(Y^{\text{LE}})^k}, \left(\sum_{k=1}^{\infty} p^{\text{LE}}(k) = 1 \right).$$

Using the above relations, the relative probability $P^{\text{LC(LE)}}(k)$ that a molecule in the LC (LE) phase is in an LC (LE) cluster of size k , is equal to $k p^{\text{LC(LE)}}(k) / \langle k^{\text{LC(LE)}} \rangle = k (Y^{\text{LC(LE)}} - 1)^2 / (Y^{\text{LC(LE)}})^{k+1}$.

Results and discussion

π - A isotherms and cluster size distributions

Surface pressure (π) - molecular area (A) isotherms for DMPC monolayers at different temperatures are shown in Figure 1. As the subphase temperature increases, the transition region seen as the plateau appears at a higher pressure and the transition width becomes narrower, being consistent with previous studies.^{38,39} We can estimate the area and the surface pressure of the LE boundary from the tangent method easily and obtain $(A_{\text{LE}}, \pi_{\text{LE}}) = (0.605 \text{ nm}^2, 12.5 \text{ mN/m})$ and $(0.514 \text{ nm}^2, 27.0 \text{ mN/m})$ at $T = 9$ and 15 °C, respectively. The symbols β and δ on isotherm curves indicate the LC boundary point $(A_{\text{LC}}, \pi_{\text{LC}})$ determined by the deconvolution procedure (see Figures 3 and 4). For comparison the deconvolution procedure has been made also using the values of LC phase boundaries (α and γ) estimated by the conventional extrapolation. ΔA_{LC} indicates the difference $\Delta A_{\text{LC}} = A_{\text{LC}}^{\text{deconv}} - A_{\text{LC}}^{\text{ext}}$ between the $A_{\text{LC}}^{\text{deconv}}$ (β or δ) determined by the deconvolution procedure and the extrapolated LC area $A_{\text{LC}}^{\text{ext}}$ (α or γ). Figure 2 shows the isothermal lateral compressibilities $\kappa_T^A(\pi) = -(1/A) \cdot (\partial A / \partial \pi)_T$ calculated from the isotherms shown in Figure 1. The transition midpoint surface pressure π_m (, defined as the position of the maximum in $\kappa_T^A(\pi)$) at each temperature can be evaluated easily

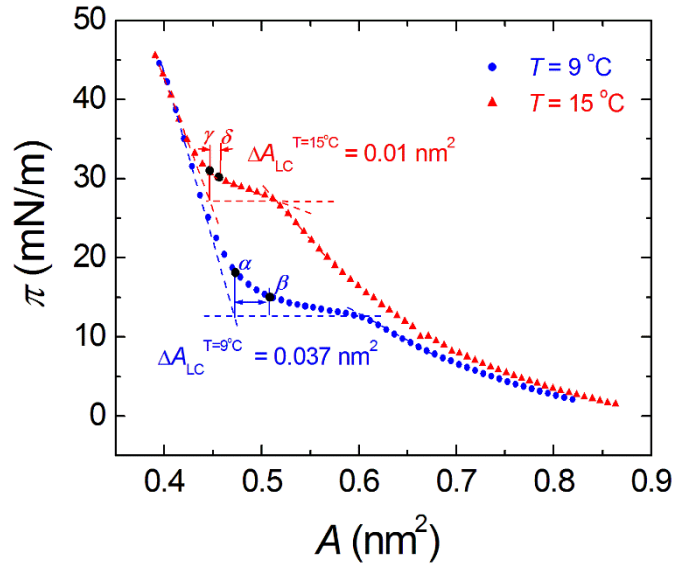


Figure 1 Surface pressure (π) – molecular area (A) isotherms for DMPC monolayers at 9 °C and 15 °C. The dashed lines indicate how the extrapolation (tangent) method was used to estimate the extrapolated area A_{LC}^{ext} (α or γ). ΔA_{LC} indicates the difference ($\Delta A_{LC} = A_{LC}^{deconv} - A_{LC}^{ext}$) between the area A_{LC}^{deconv} (β or δ) determined by the deconvolution procedure and the extrapolated area A_{LC}^{ext} (α or γ).

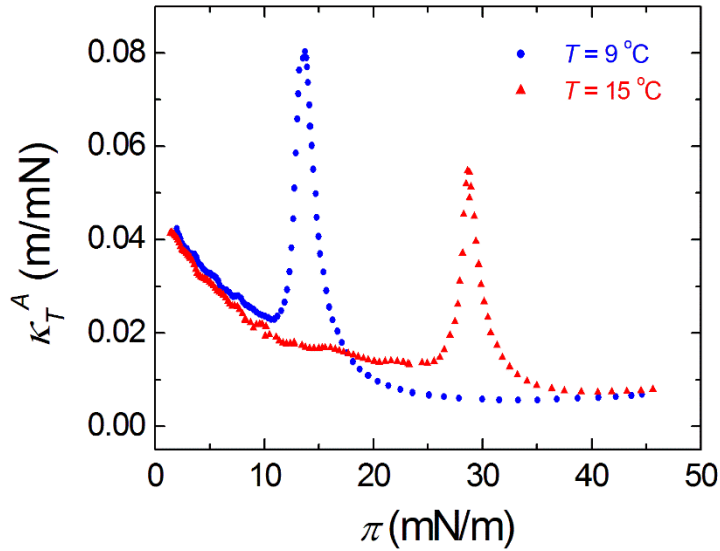


Figure 2 Isothermal lateral compressibility (κ_T^A) - surface pressure (π) characteristics for DMPC monolayers at different temperatures. A pronounced peak can be seen in each curve.

from this figure ($\pi_m^{T=9^\circ C} = 13.7$ mN/m; $\pi_m^{T=15^\circ C} = 28.6$ mN/m). The average molecular cluster

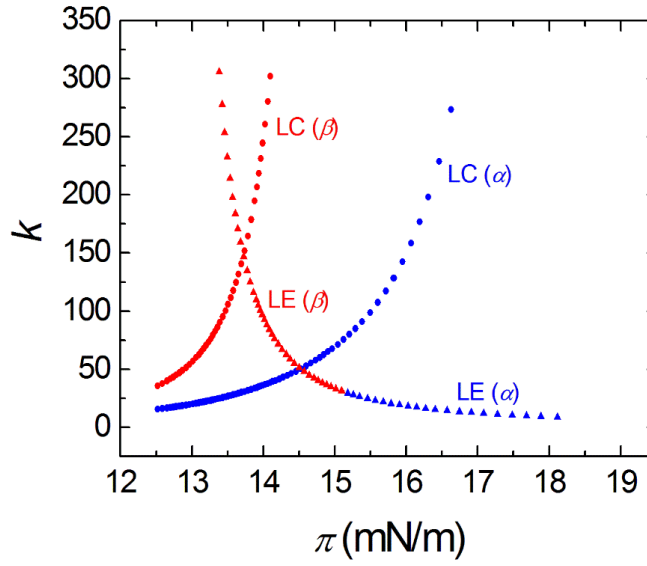


Figure 3 The average molecular cluster size k (in units of numbers of molecules) as a function of surface pressure π in the transition region of DMPC monolayer at 9 °C. All curves were calculated by the deconvolution procedure (Eqs. 1 and 2) from the isotherm shown in Figure 1. ● (LC clusters); ▲ (LE clusters). The symbols α, β correspond to the points shown on the isotherm at 9 °C in Figure 1. (α) (A_{LC}, π_{LC}) = (0.472 nm², 18.1 mN/m); (β) (A_{LC}, π_{LC}) = (0.508 nm², 15.1 mN/m). Note that the area of the point α corresponds to the extrapolated A_{LC}^{ext} from the isotherm.

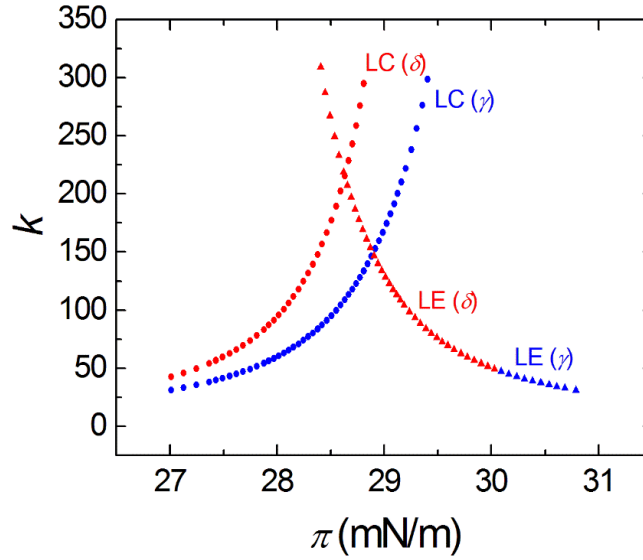


Figure 4 The average molecular cluster size k (in units of numbers of molecules) as a function of surface pressure π in the transition region of DMPC monolayer at 15 °C. All curves were calculated by the deconvolution procedure (Eqs. 1 and 2) from the isotherm shown in Fig. 1. ● (LC clusters); ▲ (LE clusters). The symbols γ, δ correspond to the points shown on the isotherm at 15 °C in Figure 1. (γ) (A_{LC}, π_{LC}) = (0.447 nm², 30.9 mN/m); (δ) (A_{LC}, π_{LC}) = (0.456 nm², 30.1 mN/m). Note that the area of the point γ corresponds to the extrapolated A_{LC}^{ext} from the isotherm.

distributions obtained by the deconvolution procedure (Eqs. 1 and 2) using the isotherm data at 9 °C and 15 °C are shown in Figures 3 and 4, respectively. The symbols α , β and γ , δ in each figure correspond to those shown in Figure 1. The LE and LC cluster distribution curves (β , δ) have been obtained such that the surface pressure at which they intersect to each other can coincide with that at which the compressibility curve exhibits a peak. The use of the extrapolated boundary area A_{LC}^{ext} and the corresponding surface pressure π_{LC}^{ext} (i.e., those of the point α or γ) on each isotherm curve in the deconvolution process leads to the intersection (transition midpoint) of LE and LC cluster curves at a higher surface pressure compared to the surface pressure at which the compressibility peak appears. Figure 5 shows the comparison of the average cluster size distributions as a function of the rescaled surface pressure π/π_m at different temperatures. As the temperature approaches the critical temperature $T_c (\approx 20 \text{ }^\circ\text{C})$ of DMPC monolayer,³⁹ the cluster size at the transition midpoint becomes larger and the full width at half maximum (FWHM) of the peak decreases. In Figure 6 we show the relative probability $P(k)$ at the transition midpoint as a function of cluster size k . From Figures 5 and 6, the max value of P occurs at k_{max} (the average cluster size at π_m), however, the distribution is very broad and there are more molecules belonging to the clusters whose size are larger than the average at each temperature.

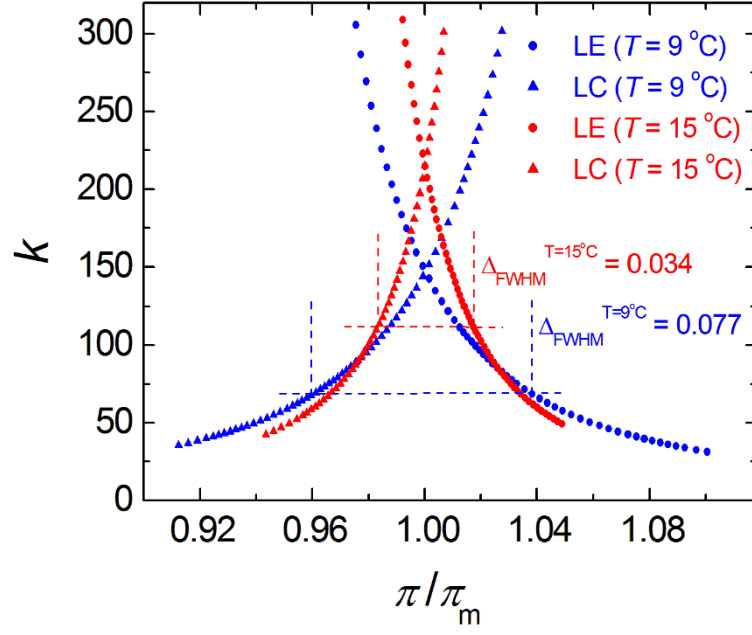


Figure 5 The average LE and LC cluster size k distributions as a function of rescaled surface pressure π/π_m at different temperatures.

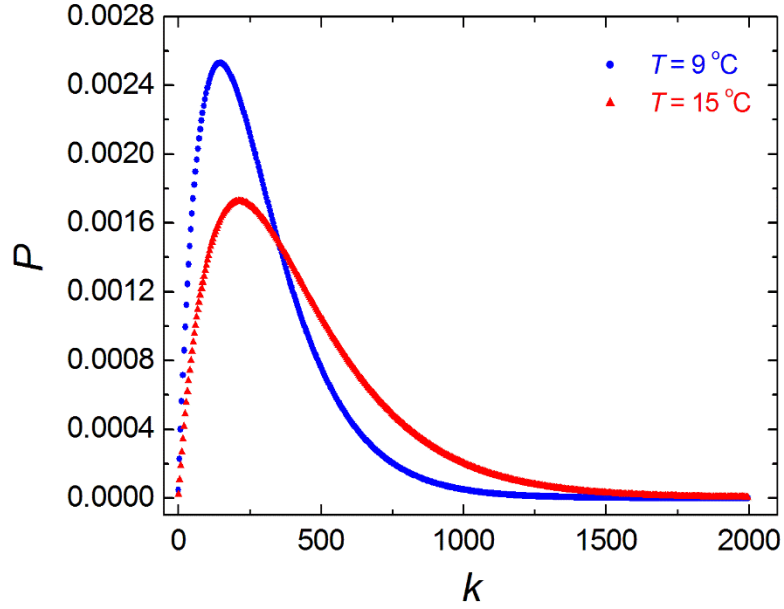


Figure 6 Relative probability P that a DMPC molecule is in a cluster of size k at the transition midpoint at different temperatures.

Deconvolution analysis for isotherms and molecular area fluctuations

The spatial density fluctuations in the phase coexistence region are microscopically characterized by a correlation length corresponding to the average linear cluster size. We note that the average cluster size is an equilibrium property although individual clusters constantly form and annihilate with a finite life-time in the thermodynamic majority phase (“dynamic heterogeneity”⁴⁰). The macroscopic manifestation of the persistence of such dynamic nanoclusters is accessible from the corresponding thermodynamic response function with the help of the equilibrium FD theorem.³³ The present deconvolution scheme has been developed by utilizing the close relation via the equilibrium FD theorem between microscopic molecular area (density) fluctuations characterized by average cluster sizes and macroscopic lateral compressibility calculated from isotherm. Such a fluctuation-based approach seems to be more effective for the analysis of phase transitions in monolayer as a two-dimensional system since from the Ginzburg criterion a wider temperature region for critical behavior is expected than in three dimensions.⁴¹ The critical behavior from the thermodynamic as well as the structural data in lipid monolayers was found to be consistent with the 2D Ising universality class.³⁹ In this class the observables such as correlation length ξ and line tension λ follow general power laws and scaling relations, $\xi \propto |(T - T_c)/T_c|^{-\nu}$ and $\lambda \propto |(T - T_c)/T_c|^\mu$.⁴² The critical exponent for line tension μ is related to that for correlation length ν through the number of dimensions in the system d , ($\mu = (d - 1)\nu$). For our lipid monolayer ($d=2$), $\mu = \nu$, so we have $\lambda \propto \xi^{-1}$. As seen from Figure 5, the peak intensity (I_p , defined as the crossover point of LE and LC distribution curves) of the average

cluster size distribution at the transition midpoint ($\pi/\pi_m = 1$) increases as T approaches the critical temperature T_c . Taking account that the average cluster size is a measure of the correlation length that describes the range of the correlations of the fluctuations, Figure 5 and the above inverse relation between λ and ξ indicates the decrease of λ between LE and LC phase boundary as T approaches T_c . This seems to be consistent with previous fluorescence microscopy observations in which the LC domain shapes became less progressively compact on compression with increasing temperature.⁴³ The lowering of λ at higher temperature would facilitate the wider distribution of cluster size. From the above argument it seems plausible that the clusters of different sizes are more uniformly distributed as well as the tail of the distribution extends to larger cluster size (the enhancement of density fluctuations) at the higher temperature (Figure 6).

Comparison of cluster sizes and consistency

Let us compare the cluster sizes obtained from the deconvolution analysis with those reported in the previous experimental studies on monolayers and bilayers. We have obtained $k \approx 147$ ($A_{\text{cluster}} \approx 81 \text{ nm}^2$ and $D_{\text{cluster}} \approx 10 \text{ nm}$) and $k \approx 216$ ($A_{\text{cluster}} \approx 105 \text{ nm}^2$ and $D_{\text{cluster}} \approx 12 \text{ nm}$) at the transition midpoint at 9 °C and 15 °C, respectively. Here, the average cluster area A_{cluster} and the average cluster diameter (or linear cluster size) D_{cluster} were calculated from the relationships $A_{\text{cluster}} \approx k \cdot a_{\text{mid}}$ and $D_{\text{cluster}} \approx (A_{\text{cluster}}/\pi)^{1/2}$ where a_{mid} is the molecular area at the transition midpoint. From Figures 1 and 2 one can obtain $a_{\text{mid}} = 0.554 \text{ nm}^2$ and 0.487 nm^2 at 9 °C and 15 °C, respectively. In relation to

the limited cooperativity of molecules in monolayers at the air-water interface, it was suggested that the non-horizontal slope in the isotherm for DPPC monolayers results from the finiteness of the transition involving the cooperative clusters of about 60 – 190 molecules from the van't Hoff analysis using the isotherm.²² Electron microscopy and diffraction study of phospholipid monolayers transferred from water surface to solid substrates showed the coexistence of LE and LC domains with correlation length of some 10 nm, corresponding to the linear cluster size.¹⁴ AFM was applied for the main transition of supported DMPC bilayers as a quantitative structural calorimetry from a specific image analysis and provided a cooperative cluster size of 195 molecules ($\approx 117 \text{ nm}^2$, assuming an average interfacial area of 0.6 nm^2 per lipid molecule of the bilayer).¹⁸ In calorimetric studies the size of a cooperative cluster of the lipid bilayer undergoing chain melting transition was estimated by the ratio of enthalpy derived from the van't Hoff enthalpy to the corresponding calorimetric enthalpy. The gel-liquid crystalline transition in dilute aqueous suspensions of DMPC bilayers gave $k \approx 200$.⁴⁴ In infrared spectroscopy of DMPC/water multi-bilayers, the van't Hoff enthalpy of the main transition was estimated from the temperature dependence of the transmittance of specific absorption bands and the ratio of this value to the previously reported calorimetric enthalpy⁴⁵ gave $k \approx 90\text{-}120$.⁴⁶ The temperature dependence of Raman scattering intensity of a spectral feature that depends upon the distribution between two states of the molecules was shown to provide the effective cluster size, k_{eff} ⁴⁷ and the analysis of the main gel to liquid crystalline transition for multi-lamellar dispersions of DPPC gave $k_{\text{eff}} \approx 100$. From the above, our deconvolution results seem to be generally consistent with

previously reported results. It should be noted that the present procedure will be much simpler compared to the other methods that have been made.

LC phase boundary area

We can see from Figure 1 that for both temperatures measured there is a finite difference $\Delta A_{\text{LC}} = A_{\text{LC}}^{\text{deconv}} - A_{\text{LC}}^{\text{ext}} (\neq 0)$ between the values of the LC boundary area determined from each of the deconvolution procedure and the conventional extrapolation method and that ΔA_{LC} decreases significantly with increasing temperature. We consider this finally. From the application of the lever rule for the combined BAM/isotherm results of DPPC monolayers, Arriaga et al. showed that the phase boundary areas deduced from the molecular area dependence of the LC phase area fraction correspond well to those estimated by the conventional extrapolation to isotherm.²⁸ The validity of the application of the lever rule based on the image analysis for the determination of the phase boundary was confirmed also for monolayers of pentadecanoic acid (PDA)⁷ and L- α -dimyristoyl phosphatidic acid (DMPA)⁴⁸ with FM/isotherm measurements. We must note, however, that the above lever rule analysis is valid only in the case that the area fraction of the micrometer-sized LC domains can be an accurate measure for tracking the progress of the transition. Using fluorescence microscopy and spectroscopy for DMPE monolayers, Flörsheimer and Möhwald indeed observed that the area fraction of LC phase domains as a function of molecular area extrapolates to zero for a smaller area than the one corresponding to a break in the isotherm slope.²⁹ Importantly they found from the measurements of

the fluorescence emission intensity profile that i) the onset of the transition is deduced before LC phase domains are microscopically visible and ii) its onset area corresponds well to the onset of the phase transition as obtained from the conventional extrapolation. They ascribed these findings to the formation of two-dimensional microscopically invisible clusters as examined in our present study that at higher surface pressures (or at smaller areas) aggregate to form microscopically visible domains (*a two-stage growth process*). From thermodynamic and energetic considerations Israelachvili suggested that, with increasing surface pressure in monolayers surfactant molecules should first nucleate into nanodomains with aggregation numbers *in the tens to hundreds* in the coexistence region before they associate into larger structures such as 2D mesophases as observed by optical microscopes at higher pressures.⁴⁹ Besides these experimental²⁹ and theoretical⁴⁹ investigations, Peters and Beck observed that the LE-LC transition of the lipid monolayer is accompanied by sudden reduction to $<10^{-3}$ of the translational diffusion constant in the phase coexistence region with increasing surface pressure.⁵⁰ This remarkable slowing down of the diffusion of molecules in the coexistence region may be closely related to the slow hierarchical (two-stage) growth of monolayer. If cooperative clusters comprising of $\approx 10^2$ molecules *retain* a high diffusional mobility in the coexistence region different from the case of the two-stage growth scenario, they will aggregate quickly to form optically visible domains made up of $\approx 10^6$ molecules without a time delay (*a direct growth process*). Concerning the LC phase boundary of DPPC monolayers, it was reported that the boundary area deduced from the lever rule analysis is normally much smaller or at largest approximately equal to that determined from the conventional

extrapolation.^{30,31,32} It was shown also for the LE phase boundary that the boundary area deduced from the lever rule analysis is rather smaller than that corresponding to the abrupt change in the slope of isotherm and that the difference between them clearly tends to decrease with increasing temperature.³⁰ Along the two-stage growth scenario, considering the above previous results^{30,31,32} and the temperature dependence of ΔA_{LC} in our deconvolution analysis together, the LC boundary areas deduced from each of the area ratio of *mesoscopic* LC domains (lever rule analysis) and the *nanoscopic* cluster distribution (deconvolution analysis) seem to approach each other from opposite sides with increasing temperature, getting very close to the value determined by the tangential extrapolation to the isotherm curve in the limit of absence of time delay during the hierarchical growth process. Apparent violation of the lever rule based on the image analysis on the specific length scales (several tens to hundreds of microns) in lipid monolayers would be a natural consequence from the occurrence of slow hierarchical aggregation between different length scales. It is well known that the shape of the isotherm in the LE-LC coexistence region is generally strongly anisotropic at low temperatures with a rounded high- π shoulder and a distinct kink at the low- π side and that its shape asymmetry becomes less prominent as the temperature increases. It would also be interesting to examine this behavior from the viewpoint of slow hierarchical growth scenario.

Conclusions

A statistical mechanical deconvolution analysis to monolayer isotherm has been performed combined

with isothermal compressibility data to obtain some information on the formation and aggregation of clusters in the nm range in the phase coexistence region of the first-order LE-LC transition for DMPC monolayers at the air-water interface. The present methodology is based on the close relationship via the fluctuation - dissipation theorem in equilibrium between isothermal compressibility as a macroscopic thermodynamic response function and size fluctuations of nanoscopic clusters and utilizes the fact that for a surface pressure-driven diffuse transition the transition midpoint can be determined macroscopically from the position at which $\kappa_T^A(\pi)$ shows a peak as well as microscopically from that at which LE and LC cluster curves cross. Our approach can make it possible from a single isotherm to simultaneously determine (i) nanoscopic LE and LC cluster size distributions in the coexistence region and (ii) LC phase boundary (A_{LC} , π_{LC}). Although the LC phase boundary has so far been determined less definitely by the tangential extrapolation of isotherm curve, the present deconvolution scheme allows one to determine it uniquely, without ambiguity. The values of the cluster sizes obtained from the present method seem to be well correlated with those reported by previous experimental studies, such as TEM^{14,15}, AFM¹⁵⁻²¹, scanning calorimetry⁴⁴, infrared spectroscopy⁴⁶, and Raman spectroscopy⁴⁷. The cause for the finite difference between the LC boundary areas obtained from each of the deconvolution procedure and the conventional extrapolation method to the same isotherm seems to be ascribed to slow hierarchical growth from nanoscopic clusters to meso- and even macroscopic domains, however, a further examination on this point must be made. The present study suggests that it is significant to complementarily investigate monolayer growth across different length

scales simultaneously for the deeper understanding of the first order phase transition of monolayers at the air-water interface. As an extension of the present work, the application of the deconvolution scheme to first order transitions in monolayers of lipid/cholesterol and binary lipid mixtures may provide some valuable information about the nanoscopic cluster formation and aggregation in the phase coexistence of complex model membrane systems.

Author contributions

K.N. performed all the experiments. E.H. supervised the experimental work. E. H. performed the deconvolution formulation. All authors analyzed the results and contributed to writing and editing the manuscript.

References

- 1 G. L. Gaines, *Insoluble monolayers at liquid-gas interfaces*, Wiley, New York, 1966.
- 2 V. M. Kaganer, H. Möhwald, P. Dutta, *Rev. Mod. Phys.*, 1999, **71**, 779-819.
- 3 V. von Tscharner, H. M. McConnell, *Biophys. J.*, 1981, **36**, 409-419.
- 4 M. Lösche, E. Sackmann, H. Möhwald, *Ber. Bunsenges. Phys. Chem.*, 1983, **87**, 848-852.
- 5 D. Hönig, D. Möbius, *J Phys. Chem.*, 1991, **95**, 4590-4592.
- 6 S. Hénon, J. Meunier, *Rev. Sci. Instr.*, 1991, **62**, 936-939.
- 7 B. G. Moore, C. M. Knobler, S. Akamatsu, F. Rondelez, *J. Phys. Chem.*, 1990, **94**, 4588-4595.
- 8 S. Hénon, J. Meunier, *J. Chem. Phys.*, 1993, **98**, 9148-9154.
- 9 C. M. Knobler, in *Advances in Chemical Physics*, Wiley, New York, 1990, **77**, 397-449.
- 10 H. Möhwald, in *Handbook of Biological Physics*, Vol. I, Elsevier, Amsterdam, 1995, 161-211.
- 11 E. Hatta, K. Nihei, *Phys. Rev. E.*, 2019, **100**, 0228011-02280110.
- 12 Ole G. Mouritsen, in *Molecular Description of Biological Membrane Components by Computer Aided Conformational Analysis*, Vol. I, CRC Press, Boca Raton, Florida, 1990, 3-83.
- 13 Ole G. Mouritsen, J. H. Ipsen, M. J. Zuckermann, *J. Colloid Interface Sci.*, 1989, **129**, 32-40.
- 14 A. Fischer, E. Sackmann, *J. Physique*, 1984, **45**, 517-527.
- 15 F. Boury, A. Gulik, J. C. Dedieu, J. E. Proust, *Langmuir*, 1994, **10**, 1654-1656.
- 16 L. F. Chi, M. Anders, H. Fuchs, P. R. Johnston, H. Ringsdorf, *Science*, 1993, **259**, 213-216.
- 17 X-M. Yang, D. Xiao, S-J. Xiao, Z-H. Lu, Y. Wei, *Phys. Lett. A*, 1994, **193**, 195-198.
- 18 O. Enders, A. Ngezahayo, M. Wiechmann, F. Leisten, H. –A. Kolb, *Biophys. J.*, 2004, **87**, 2522-2531.
- 19 A. Angelova, D. Vollhardt, R. Ionov, *J. Phys. Chem.*, 1996, **100**, 10710–10720.
- 20 A. Angelova, M. Van der Auweraer, R. Ionov, D. Vollhardt, F. C. De Schryver, *Langmuir*, 1995, **11**, 3167–3176.
- 21 A. Angelova, D. Vollhardt, R. Ionov, *Thin Solid Films*, 1996, **284-285**, 85-89.

-
- 22 O. Albrecht, H. Gruler, E. Sackmann, *J. Phys. (Paris)*, 1978, **39**, 301-313.
- 23 E. Hatta, T. Nishimura, *J. Colloid Interface Sci.*, 2013, **391**, 111-115.
- 24 E. Freire, R. L. Biltonen, *Biopolymers*, 1978, **17**, 481-496.
- 25 S. Akamatsu, F. Rondelez, *J. Phys. II France*, 1991, **1**, 1309-1322.
- 26 B. Y. Yue, G. M. Jackson, J. A. G. Taylor, J. Mingins, B. A. Pethica, *J. Chem. Soc., Faraday Trans. 1*, 1976, **72**, 2685-2693.
- 27 T. L. Hill, *Thermodynamics of Small Systems*, Part 1, W.A. Benjamin, New York, 1963.
- 28 L. R. Arriaga, L. Lopez-Montero, J. Iñes-Mullol, F. Monroy, *J. Phys. Chem. B.*, 2010, **114**, 4509-4520.
- 29 M. Flörsheimer, H. Möhwald, *Thin Solid Films*, 1988, **159**, 115-123.
- 30 M. Flörsheimer, H. Möhwald, *Chem. Phys. Lipids*, 1989, **49**, 231-241.
- 31 K. Nag, C. Boland, N. Rich, K. M. W. Keough, *Biochim. Biophys. Acta*, 1991, **1068**, 157-160.
- 32 K. J. Klopfer, T. K. Vanderlick, *J. Colloid Interface Sci.*, 1996, **182**, 220-229.
- 33 L. E. Reichl, *A Modern Course in Statistical Physics*, 4th ed., John Wiley and Sons, New York, 2016.
- 34 E. Di Cera, *Thermodynamic Theory of Site-Specific Binding Processes in Biological Macromolecules*, Cambridge University Press, Cambridge, 1995, 1-46.
- 35 Ole G. Mouritsen, M. J. Zuckermann, *Chem. Phys. Lett.*, 1987, **135**, 294-298.
- 36 J. H. Ipsen, K. Jørgensen, Ole G. Mouritsen, *Biophys. J.*, 1990, **58**, 1099-1107.
- 37 E. Freire, R. L. Biltonen, *Biochim. Biophys. Acta.*, 1978, **514**, 54-68.
- 38 D. Steppich, J. Griesbauer, T. Frommelt, W. Appelt, A. Wixforth, M. F. Schneider, *Phys. Rev. E.*, 2010, **81**, 0611231-0611235.
- 39 L. K. Nielsen, T. Bjørnholm, O. G. Mouritsen, *Langmuir*, 2007, **23**, 11684-11692.
- 40 D. B. Mountcastle, R. L. Biltonen, M. J. Halsey, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 4906-4910.
- 41 J.-C. Tolédano, P. Tolédano, *The Landau Theory of Phase Transitions: Application to Structural, Incommensurate, Magnetic and Liquid Crystal Systems*, World Scientific, Singapore, 1987.
- 42 N. Goldenfeld, *Lectures on Phase Transitions and the Renormalization Group*, Addison-Wesley, New York, 1992.

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- 43 K. J. Stine, D. T. Stratmann, *Langmuir*, 1992, **8**, 2509-2514.
- 44 H. -J. Hinz, J. M. Sturtevant, *J. Biol. Chem.*, 1972, **247**, 6071-6075.
- 45 D. Chapman, R. M. Williams, B. D. Ladbroke, *Chem. Phys. Lipids*, 1967, **1**, 445-475.
- 46 A. Mellier, J. Kanza, *J. Chim. Phys.*, 1993, **90**, 51-62.
- 47 W. H. Kirchhoff, I. W. Levin, *J. Res. Natl. Bur. Stand. (U. S.)*, 1987, **92**, 113-128.
- 48 M. Lösche, H. -P. Duwe, H. Möhwald, *J. Colloid Interface Sci.*, 1988, **126**, 432-444.
- 49 J. Israelachvili, *Langmuir*, 1994, **10**, 3774-3781.
- 50 R. Peters, K. Beck, *Proc. Natl. Acad. Sci. USA*, 1983, **80**, 7183-7187.