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Polylipids anchored to membranes:
Modulated phases and collapse

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Abstract. – Phase separation in membranes incorporating components functionalized with water soluble polymers exhibits novel features. These occur because the domain rich in functionalized components is decorated by a polymeric brush. Three main scenarios may occur; i) phase separation with a lowered interfacial free energy between the domains, ii) formation of modulated phases, iii) repression of the phase separation. While the discussion focuses on the collapse behavior of a single polylipid anchored to a membrane, the conclusions apply to a wide variety of systems.

Membranes incorporating anchored polymers play a role in both biological systems and biotechnology applications. Examples of the first category include the Glycocalix and the Cytoskelton [1]. "Stealth" liposomes decorated by terminally anchored poly(ethylene oxide), which are used as drug delivery vehicles, exemplify the second category [2]. Experimental studies demonstrated that polysoaps anchored to membranes can induce morphological changes in giant liposomes [3,4]. These observations motivated theoretical studies of membranes decorated by anchored polymers [5–7]. These studies concern the effect of the anchored chains on the elasticity of the membrane. They focus on the case of singly anchored chains where the anchor is compatible with the phospholipids forming the membrane.

In the following we extend these theoretical considerations to allow for two additional factors: i) Anchors that are *incompatible* with the phospholipids forming the membrane. ii) Polymers incorporating *multiple anchors* that are joined by flexible and water-soluble spacer chains. As we shall see, phase segregation in this system is associated with frustration leading to a novel phase diagram. When the anchors are inserted into the membrane the spacer chains joining them form loops that are grafted to the membrane. Segregation of the incompatible anchors decreases the area per loop thus causing crowding, *i.e.*, the demixing is associated with the formation of a brush [8]. In turn, the crowding of the loops within the brush gives rise to a free-energy penalty that opposes the segregation of the anchors. When the spacers are long, this penalty can suppress the demixing altogether. For shorter spacers the system resolves the frustration by forming a variety of *modulated* phases. These are reminiscent of the modulated phases formed in single-component Langmuir monolayers [9]. In this last case the modulation is the result of long-ranged electrostatic interactions. In marked contrast, in

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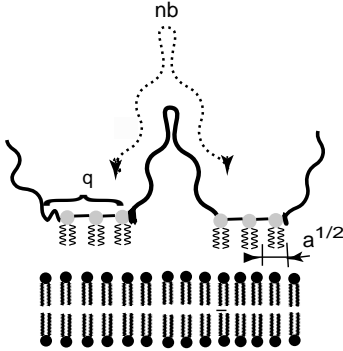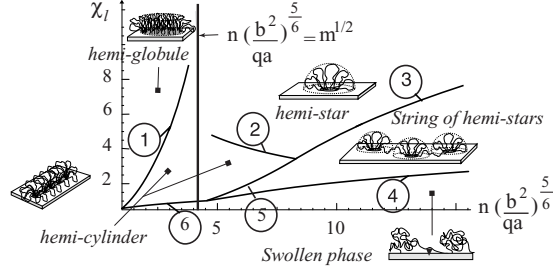(*) Also at the Institut Universitaire de France.

Fig. 1

Fig. 2

Fig. 1 – Schematic view of the architecture of a polylipid with a block structure involving m blocks, each comprising q phospholipids joined by a flexible spacer chain consisting of n monomers.

Fig. 2 – The state diagram of a single polylipid anchored to a membrane as a function of χ_1 and of n as calculated for $m = 100$, $q = 3$ and $b^2/a = 0.15$. The curves depict the crossovers obtained in the text.

the present case the modulation arises because of short-ranged interactions between the loops forming the brush.

Our discussion concerns polylipids that is, polymers incorporating covalently bound phospholipid monomers (fig. 1). In particular, it deals with multiblock copolymers comprising m phospholipid blocks consisting each of q phospholipid monomers. In the following we assume that the lipid blocks are flexible. The lipid blocks, the anchors, are joined by flexible, neutral and water-soluble blocks incorporating n monomers of size b. The chain is assumed to incorporate a single type of phospholipid denoted by α. The membrane considered is fluid, comprising a single phospholipid, β, such that α and β are incompatible. The interactions between the α and β phospholipids are characterized by $kT\chi_1$ of order of few kT [10]. Water is assumed to be an athermal good solvent for the spacer chains, joining the α blocks, i.e., the corresponding Flory interaction parameter is $\chi = 0$. For simplicity it is further assumed that the head-group areas of the α and β lipids are identical, a. Typically, a varies in the range of 50–70 Å^2 [11]. The α blocks are presumed embedded in the β membrane thus providing anchors to the chain. It is assumed that all the α blocks are embedded and that there is no exchange with the bulk. The lateral mobility within the membrane enables phase separation of the α and β lipids. The $\alpha\beta$ phase separation thus gives rise to the formation of dense α domains decorated by a brush. The nature of the resulting phases depends primarily on the values of n and q, that determine the magnitude of the brush penalty, and the incompatibility of α and β as specified by χ_1. In general, the $\alpha\beta$ phase separation should couple to the elasticity of the membrane and give rise to morphological changes. At this early stage we mostly ignore this effect and limit the discussion to a planar membrane. For brevity we focus the discussion on the two-dimensional collapse of a *single* polylipid chain, see fig. 2. It is important to note that the modulated structures that appear in a single collapsed polylipid also appear in the many-chain system and that the same control parameters are involved([1]). Furthermore, while this letter is concerned with the case of polylipids, the discussion applies, with some modification, to a much wider class of systems. One such system are membranes

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([1])This is ensured by the equality of the chemical potential of the isolated chains and the dense phase that coexist with them.

incorporating lipids functionalized by a single chain, $m = 1$, $q = 1$. Since q allows to tune the size of the anchors, it also allows to capture the essential physics of membranes incorporating polymers anchored to membrane proteins. This parameter plays an important role in our discussion because the brush penalty suppresses the $\alpha\beta$ phase separation for $q = 1$. Note that the synthesis of polysoaps with a block structure similar to the one postulated above has been reported [12]. Our discussion begins with the collapse of a polylipid into a uniform hemi-globule, neglecting boundary effects. Next, we allow for the effect of the brush on the line tension of finite hemi-globule. The so-called overspill effect [13] can lower the line tension to zero, a state defining the stability limit of this configuration. The modulated configurations that replace the hemi-globule are analyzed next by comparing the free energies of the various possible structures. In every case, the free energies of the brush component are based on the blob picture [8]. The role of exchange attractions on the chain configurations, and on the phase behavior in general, is then briefly discussed. We finally comment on aspects of the coupling between the $\alpha\beta$ phase separation and the elasticity of the membrane.

It is helpful to begin with the simplest situation involving the two-dimensional collapse of a single polylipid into a hemi-globule with a uniform density of α lipids and radius which is much larger than the thickness of the brush, L_0. We initially ignore the role of the line tension associated with the boundary of the hemi-globule. The collapsed configuration is determined by two terms in the interaction free energy of the chain. One is the mixing free energy per site $F_{\mathrm{mix}}/kT = \chi_1\phi(1 - \phi) + (1 - \phi)\ln(1 - \phi)$, where $\phi = mqa/R^2$ is the area fraction of the α lipids. The translational term, $(\phi/mq)\ln\phi$, was deleted because it does not play a role in the single-chain problem. The second term is the free-energy penalty per site due to the formation of a planar brush $F_{\mathrm{brush}}/kT \approx (n/q)(b^2/qa)^{5/6}\phi^{11/6}$. The last term is obtained from the free energy per loop $nkT(b^2/\Sigma)^{5/6}$ where the area per loop is $\Sigma = R^2/m = qa/\phi$ and the number of sites per loop is $\Sigma/a = q/\phi$. It is instructive to consider the virial expansion of $F_{\mathrm{int}} = F_{\mathrm{mix}} + F_{\mathrm{brush}}$

$$\frac{F_{\mathrm{int}}}{kT} \approx v\phi^2 + w\phi^3 + \frac{n}{q}\left(\frac{b^2}{qa}\right)^{5/6}\phi^{11/6}\,,\tag{1}$$

where $v = kT(1 - 2\chi_1)$ is the second virial coefficient and $w = 1/3$ is the third virial coefficient. The density within the globule is determined by the condition that the osmotic pressure of the monomer vanishes, $\pi = 0$ [14]. As in the familiar scenarios this corresponds to $\phi \approx w/|v|$, *i.e.*, the binary monomer-monomer attraction is balanced by ternary repulsions. However, in our case, the brush term gives rise to an activation barrier at $\phi \approx n^6|v|^{-6}q^{-11}(b^2/a)^5$. As stated earlier, this analysis overlooks the role of boundary effects and their influence on the line tension. At the edge of the hemi-globule the loops forming the brush spill sideways thus lowering their crowding [13]. This overspill effect gives rise to a negative correction to the line tension, τ. The effective τ reflects two contributions: One, $\tau_1 \geq 0$, is due to the interaction between the incompatible lipids. The correction due to the overspill effect, τ_{os}, may be estimated by considering the work done by the system as the brush expands sideways. A brush element of unit width is assumed to expand so as to occupy a volume element of cross-section L_0^2, where $L_0/b \approx n(b^2/\Sigma)^{1/3}$ is the equilibrium height of the brush. This leads to the expenditure of πL_0^2, where the osmotic pressure is estimated by $\pi \approx kT/\xi^3$ and $\xi \approx \Sigma^{1/2}$ is the blob size within the Alexander model. For brevity we limit the discussion to strongly incompatible α and β such that $\phi \simeq 1$ and $\Sigma^{1/2} \approx \xi \simeq (qa)^{1/2}$. In this case we may estimate τ_1 by $\tau_1/kT \approx \chi_1/a^{1/2}$, thus leading to

$$\frac{\tau a^{1/2}}{kT} \approx \chi_1 - \frac{n^2}{q^{1/2}}\left(\frac{b^2}{qa}\right)^{5/3}\,.\tag{2}$$

While $\tau > 0$ the hemi-globule adopts a circular form and the radius of the α domain is $(mqa)^{1/2}$. However, τ decreases as n increases leading, eventually, to $\tau = 0$ when $\chi_1 \sim n^2/q^{13/6}$. This condition specifies the limit of stability of the hemi-globule since when $\tau < 0$ it is beneficial to increase the length of the boundary indefinitely. This argument cannot however specify the equilibrium structures that replace the hemi-globule because the creation of longer boundary gives rise to small α domains for which the planar brush model is no longer applicable.

To specify the equilibrium structures that replace the unstable hemi-globule it is necessary to compare the free energies of the different possible chain configurations. In the following we will analyze the following chain configurations: i) hemi-cylinder with a rectangular α domain of length H and width W supporting a hemi-cylindrical corona; ii) a string of $p \geq 1$ hemi-stars each with an α domain of radius $R \approx (mqa/p)^{1/2}$ smaller than the span of the corona; iii) a swollen chain where the brush penalty repressed the collapse altogether. The equilibrium states reflect an interplay of two contributions. One is the free energy of the $\alpha\beta$ boundary. It increases roughly along the sequence: hemi-star, hemi-cylinder, string of hemi-stars, swollen chain. The second is the brush penalty. Its magnitude diminishes as the volume available to a loop increases. It thus decreases along the sequence: cylinder, star, string of stars, swollen chain.

The free energy of a collapsed chain in the hemi-cylindrical form reflects two contributions. The $\alpha\beta$ boundary gives rise to a term of the form $(W + H)\tau_1$, where $WH \approx mqa$. The coronal free energy per loop, when W is small compared to the width of the corona, is $n^{3/8}(mb/H)^{5/8}kT$ [8]. The free energy of the hemi-cylindrical form is thus $F_{\text{cyl}}/kT \approx m(bW/qa)^{5/8}n^{3/8} + (W + mqa/W)\chi_1/a^{1/2}$. The equilibrium condition $\partial F_{\text{cyl}}/\partial W = 0$ leads to $W/a^{1/2} \approx \chi_1^{8/13}qn^{-3/13}(b^2/a)^{-5/26}$ and thus the equilibrium free energy of the hemi-cylinder is

$$F_{\text{cyl}}/kT \approx m\chi_1^{5/13}n^{3/13}(b^2/a)^{5/26} \,. \tag{3}$$

The free energy of a string of hemi-stars reflects the same two contributions. However, the change of symmetry results in different functional forms. The boundary contribution is $\tau_1 pR$ while the coronal free energy of an individual star-like corona of thickness $L_{\text{s}}/b \approx (m/p)^{1/5}n^{3/5}$ is $kT(m/p)^{3/2}\ln L_{\text{s}}/R \approx kT(m/p)^{3/2}$ [8]. This form applies when $L_{\text{s}} \gg R$. The total free energy of the chain is thus $F_{\text{s}}/kT \approx m^{3/2}p^{-1/2} + \chi_1 m^{1/2}q^{1/2}p^{1/2}$. The equilibrium condition $\partial F_{\text{s}}/\partial p = 0$ determines the equilibrium number of hemi-stars per chain

$$p \approx \chi_1^{-1}mq^{-1/2} \tag{4}$$

and the equilibrium free energy of the chain

$$F_{\text{s}}/kT \approx \chi_1^{1/2}mq^{1/4} \,. \tag{5}$$

The free energy of the swollen case is dominated by the interactions between the α lipids anchors and their β neighbors. Each anchor block is assumed to comprise a flexible chain incorporating q α-lipids. In two dimensions the collapsed and ideal chain configurations exhibit an identical scaling behavior, and the anchor may be envisioned as an α domain of radius $r \sim (qa)^{1/2}$. The line tension associated with these domains, τ_1, determines the free energy of the swollen chain $F_{\text{sw}}/kT \approx \tau_1 r$ or

$$F_{\text{sw}}/kT \approx mq^{1/2}\chi_1 \,. \tag{6}$$

Having specified the different states of the chain we are in a position to obtain its state diagram as depicted in fig. 1. A crossover between the hemi-globule and a single hemi-star,

$p = 1$, is found by comparing (5) with the dominant term in the free energy of a dense, $\phi \simeq 1$, hemi-globule

$$F_{\mathrm{g}}/kT \approx mn(b^2/qa)^{5/6}\,. \tag{7}$$

It occurs at $m \approx n^2(b^2/qa)^{5/3}$. Upon increasing n further the single hemi-star breaks into a string of hemi-stars. The boundary of these two regimes is specified by $p = 1$ as given by (4) leading to $\chi_1 \approx mq^{-1/2}$ (line 3). Eventually, for yet larger n the collapse is repressed altogether and the chain remains swollen. The boundary of the swollen chain regime, as obtained by comparing (5) and (6), occurs at $\chi_1 \approx q^{-1/2}$ (line 4), up to logarithmic factors. At lower values of χ_1, the hemi-globule transforms into a hemi-cylinder when increasing n. The crossover, as specified by comparing (7) and (3), occurs at $\chi_1 \approx n^2 q^{13/6}(b^2/a)^{5/3}$ (line 1). Note that this boundary is identical to the stability limit obtained from (2). The boundary between the hemi-cylinders and the hemi-stars occurs when (3) equals (5) for $p = 1$ and is specified by $\chi_1 \approx m^{13/10}n^{-3/5}(b^2/a)^{1/2}$. Finally, upon increasing n further the chain assumes a swollen configuration. The boundary between the hemi-cylinder and the swollen configuration occurs at $\chi_1 \approx n^{3/8}q^{-1/2}(b^2/qa)^{5/16}$ when (3) and (6) are comparable (line 6). While crossing of free-energy curves is a rough diagnostic for a first-order phase transition, our considerations are too rough to investigate this aspect.

The analysis presented above specifies the local structure of the chain. Additional considerations are necessary in order to determine the large-scale chain configuration. Two extra ingredients are required. For the hemi-cylinders, it is necessary to determine the persistence length, l_{p}, associated with the bending modulus. It is found that $W \ll l_{\mathrm{p}} \ll H$, thus suggesting that the hemi-cylinder is semiflexible. A detailed discussion of this point is beyond the scope of this letter. The second ingredient is the effect of exchange attraction. When the coronas of two α domains are at grazing contact, the spacer chains may assume two configurations: one where both α anchors reside in the same α domain and a second, where each α anchor resides in a different domain. When the residence time is short enough, this leads to gain in free energy of roughly $kT \ln 2$ per loop, thus giving rise to an entropic attraction that favors grazing contact [15]. A more detailed analysis reveals that the resulting attraction at grazing contact of hemi-stars scales as $kTp^{1/2}$ [16]. The exchange attraction favors a collapsed configuration of the chain. For the case of a string of hemi-stars, the chain will form a circular domain of close-packed hemi-stars. The effect of the exchange attraction on the configuration of hemi-cylindrical chain requires an analysis of "hairpin defects" and is beyond the scope of this letter. Importantly, the exchange attraction is expected to lead to phase separation in many chain systems when the individual chains assume a hemi-star or a hemi-cylinder configuration.

Multicomponent membranes, comprising a mixture of different lipids or of lipids and proteins, are capable of undergoing phase separation leading to the formation of mesoscopic domains. The phase behavior is strongly modified when one of the components is attached to a water-soluble polymer. Three main scenarios are possible: i) mesoscopic phase separation with a reduced line tension between the domains; ii) formation of various modulated phases; iii) complete repression of the phase separation. Our analysis of these scenarios focused on the case of a single polylipid ($m \gg 1$) anchored to a membrane. However, with certain straightforward modifications the discussion can be applied to many-chain systems as well as to singly anchored chains ($m = 1$). For many-chain systems it is necessary to allow for the translational entropy of the chains. For singly anchored chains clearly there is no collapse and the phenomenology we discussed is only expected for many-chain systems. Importantly, for typical values of a the phase segregation is repressed whenever $q = 1$. It can only occur

when $q > 1$ and the minimal area per chain is higher thus leading to a lower brush penalty. As noted in the introduction, we limited our analysis to the case of a planar membrane and neglected any coupling to the elasticity of the membrane. To conclude our discussion we point out an immediate outcome concerning such coupling. Phase separation within the membrane was proposed as a mechanism for budding [17]. Within this model, the domain boundary is located at the neck of the bud. Accordingly, the creation of the neck shortens the length of the boundary between the domains. The consequent lowering of the boundary free energy is associated however with an elastic penalty due to the bending of the membrane. The balance of the boundary penalty, $\tau_l R$, and the elastic term, $8\pi\kappa$, determines the size of the bud which scales as the invagination length, κ/τ_l, where κ is the bending rigidity of the membrane. When one of the domains is rich in lipids functionalized by polymers, the overspill effect comes into play and τ_l is replaced by $\tau < \tau_l$ as given by (2) thus causing an increase of the invagination length.

REFERENCES

[1] LODISH H. *et al.*, *Molecular Cell Biology* (Freeman, New York) 2000.

[2] LASIC D. D., *Liposomes, from Physics to Applications* (Elsevier, Amsterdam) 1993.

[3] RINGSDORF H., SCHLARB B. and VENZER J., *Angew. Chem. Int. Ed. Engl.*, **27** (1988) 113.

[4] FRETTE V. *et al.*, *Phys. Rev. Lett.*, **83** (1999) 2465.

[5] (a) LIPOWSKY R., *Europhys. Lett.*, **30** 197 (1995); (b) HIERGEIST C. and LIPOWSKY R., *J. Phys. II*, **6** (1996) 1465; (c) BREIDENICH M., NETZ R. R. and LIPOWSKY R., *Europhys. Lett.*, **49** (2000) 431.

[6] (a) MARQUES C. M. and FOURNIER J. B., *Europhys. Lett.*, **35** (1996) 361; (b) BIKEL T., JEPPESEN C. and MARQUES C. M., *Phys. Rev. E*, **62** (2000) 1124.

[7] BIVAS I. *et al*, *Europhys. Lett.*, **41** (1998) 261.

[8] HALPERIN A., TIRRELL M. and LODGE T. P., *Adv. Polym. Sci.*, **100** (1992) 31.

[9] ANDELMAN D. *et al.*, in *Micelles, Membranes, Microemulsions and Monolayers*, edited by W. M GELBART, A. BEN-SHAUL and D. ROUX (Springer, New York) 1994 and references quoted therein.

[10] GROVES J. T., BOXER S. G. and H. M. MCCONNELL, *Proc. Natl. Acad. Sci. USA*, **95** (1998) 13390.

[11] ISRAELACHVILI J. N., *Intermolecular and Surface Forces*, 2nd edition (Academic Press, New York) 1992.

[12] STAHLER K., SELB J. and CANDAU F., *Langmuir*, **15** (1999) 7565.

[13] (a) RAPHAEL E. and DE GENNES P. G., *Physica A*, **177** (1991) 294; (b) HALPERIN A., in *Soft Order in Physical Systems*, edited by Y. RABIN and R. BRUINSMA (Plenum, New York) 1994.

[14] GROSBERG A. YU. and KHOKHLOV A. R., *Statistical Physics of Macromolecules* (AIP, New York) 1994.

[15] WITTEN T. A., *J. Phys. (Paris)*, **49** (1988) 1056.

[16] (a) BORISOV O. V. and HALPERIN A., *Macromolecules*, **29** (1996) 2612, a somewhat different result is given in SEMENOV A. N., JOANNY J. F. and KHOKHLOV A. K., *Macromolecules*, **28** (1995) 1066; (b) HALPERIN A., in *Supramolecular Polymers*, edited by A. CIFERRI (Dekker, New York) 2000.

[17] LIPOWSKY R., *J. Phys. II*, **2** (1992) 1825.