HYDROGEN BONDING INTERACTIONS OF LIPOSOMES SIMULATING CELL-CELL RECOGNITION. A REVIEW

CONSTANTINOS M. PALEOS*, DIMITRIS TSIOURVAS and ZILI SIDERATOU
Institute of Physical Chemistry, NCSR 'Demokritos', 15310 Aghia Paraskevi, Attiki, Greece
(author for correspondence, e-mail: paleos@chem.demokritos.gr, phone: +30 210 6503666, fax: +30 210 6529792)*

(Received 20 October 2002; accepted in revised form 19 March 2003)

Abstract. Amphiphiles bearing polar heads with the property to form hydrogen bond(s) exhibit unique organizational and aggregational behaviour. Thus appropriate amphiphilic molecules assemble and form liposomes, which further interact through hydrogen bonding with complementary molecules or liposomal counterparts affording larger and more elaborated aggregates. A number of examples are demonstrating the interaction mode of liposomes and of associated phenomena as related to the structural features of the supramolecular aggregates obtained. The recognition between cells incorporating recognizable amphiphiles in their membranes has shown similarities to the analogous interactions between liposomes. Thus molecular recognition between liposomes can be used in modeling recognitions occurring between cells. Designed experiments in this area can support the Lipid World Model proposed for the origin of life.

Keywords: amphiphiles, hydrogen bonding, molecular recognition, liposomes, origin of life, supramolecular structure

1. Introduction

Spontaneous enhancement of molecular complexity coupled with self-organization and specificity seem to form the basis from which life originated (Luisi *et al.*, 1999; Miller, 2002). Specifically, amphiphilic boundary structures are believed to have contributed to the emergence of life (Segré *et al.*, 2001; Ourisson and Nakatani, 1996; Lyubarev and Kurganov, 1997; Norris and Raine, 1998), as this is supported by the fact that abiotically and spontaneously formed bilayer membranes have similar structures with contemporary cells. In addition, the same amphiphilic systems form microenvironments in which small and large molecules can be compartmentalized which is another key requirement for living systems. In this connection liposomes and especially the giant ones (Käs and Sackmann, 1991; Decher *et al.*, 1990; Menger and Lee, 1995; Bradley, 1997), primarily formed from double-chain amphiphiles and exhibiting solubilization and interaction specificity, are considered as the closest analogs of biological cells primitively sharing their structure and function. The last property of functional reactivity is of extreme significance and is being governed by molecular recognition (Lichtenthaler, 1994; Koshland, 1994; Rebek, 1996). The principle of molecular recognition was

Origins of Life and Evolution of the Biosphere **34:** 195–213, 2004.

introduced by Emil Fischer as early as in 1894 and proved very fruitful in non-covalent synthesis (Whitesides *et al*., 1995; MacDonald and Whitesides, 1994; Fyfe and Stoddart, 1997; Prins *et al*., 2001) primarily involving hydrogen bonding interactions between complementary molecules.

A significant consequence of self-organization of amphiphiles to supramolecular aggregates including liposomes and micelles is the enhanced binding efficiency of functional moieties located at their surface (Kawasaki *et al*., 2001; Berti *et al*., 1997, 2000). Specifically, the binding efficiency is increasing by a factor of 10^2–10^4 each time one proceeds from isotropic to organized media i.e. from the bulk water to microscopic interfaces of micelles or liposomes and finally to air-water macroscopic interfaces which exhibit the highest organization (Onda *et al*., 1996). In addition, cooperative and multivalency effects (Mammen *et al*., 1998) are also operating, due to which the functional groups at the liposomal interface interact more effectively compared to isotropic media. Even under the disrupting action of water (Lemieux, 1996) on hydrogen bonds, amphiphilic molecules bearing hydrogen bonding moieties can form supramolecular aggregates exhibiting elaborated structures in certain cases (Paleos and Tsiourvas, 1997 and references therein; Ariga and Kunitake, 1998 and references therein). This process can be illustrated by the initial formation of complementary liposomes which further interact via hydrogen bonding at a second-stage, simulating in a way cell-cell or cell-liposome recognition (Paleos *et al*., 2001). In fact the receptors in biological cells are located at their surface at fixed spatial arrangements, facilitating interaction and adhesion with other cells (Cooper, 1997).

In this short review the role of functional groups, which render liposomes recognizable through hydrogen bonding will be established with reference to selected examples in an attempt to mimic analogous interactions occurring between cells. In the cited examples the functionalization of the external surface of liposomes is achieved at their preparation stage employing established methods (New, 1990). Parameters affecting molecular recognition of liposomes will be investigated in conjunction with the obtained supramolecular structures. Finally, in order to illustrate the approximate resemblance of liposomes to cells, the interaction of the latter will be discussed following incorporation of a synthetic recognizable amphiphile in cell membranes.

2. Interaction of Liposomes with Molecules Dissolved in Water through Hydrogen Bonding

Liposomes, which are rendered recognizable by the incorporation of amphiphiles with hydrogen bond forming groups at their polar heads, are susceptible to interact with complementary molecules dissolved in water. Molecules bearing at least two recognizable moieties can act as a ‘molecular glue’, binding liposomes together.

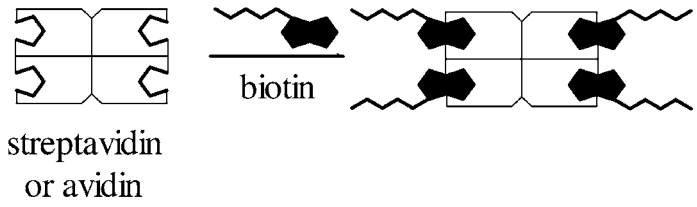


Figure 1. Interaction of biotin with the tetrameric protein streptavidin or avidin.

Typical interactions of recognizable liposomal dispersions with complementary molecules dissolved in water are discussed.

The recognizable pair of biotin (vitamin H) and streptavidin or avidin has extensively been investigated (Ahlers *et al.*, 1990) for assembling liposomes. Biotin is recognized by four identical subunits of streptavidin as shown in Figure 1, which is supported by crystallographic data (Rosano *et al.*, 1999; Freitag *et al.*, 1999; Schief *et al.*, 1999). This recognition reaction has an exceptionally high binding constant, 10^{15} M^{-1} that corresponds to a binding energy of the order of a covalent bond.

In an early example (Chiruvolu *et al.*, 1994) prepared mixed unilamellar liposomes based on dilauroylphosphatidylcholine and incorporating dipalmitoylphosphatidylethanolamine-conjugated biotin as a recognizable amphiphile. When streptavidin was added to the biotinylated liposomal dispersion, aggregation occurred followed by precipitation. The so-obtained liposomal aggregates were studied by cryoelectron microscopy, which showed that only minimal changes in liposome shape and size occurred. Most of the liposomes formed large aggregates while only few free liposomes were observed. The aggregates were bound strongly, withstanding breakage even during the relatively large shears exerted in preparing samples for cryoelectron microscopy.

Extending this work the surface of liposomes was structurally elaborated in an attempt to approximately mimic the functional behaviour of cells. Thus mixed liposomes were prepared (Noppl-Simson and Needham, 1996) by introducing biotin through the incorporation of biotin-$(CH_2)_n$-hexadecylphosphatidylethanolamine amphiphile while a repulsive steric barrier of poly(ethyleneglycol) with molecular weight 750 (PEG^{750}) was linked at the head group of another component of the liposomes, i.e., of distearoylphosphatidylethanolamine (Figure 2).

It should be noted that PEG^{750} prevented non-specific van der Walls adhesion between the two liposomes in the absence of biotin-avidin-biotin cross-bridges. Avidin-coated liposomes interacted with biotinylated liposomes leading to liposome-liposome adhesion. It was found that the rate of avidin absorption when 2 mol % PEG^{750} was introduced was four times less compared to unmodified surface while 10 mol % PEG^{750} completely suppressed binding of avidin to biotin. Also the rate of cross-bridges accumulation was consistent with the diffusion of the lipid-linked 'receptors' into the contact zone. PEG^{750} did not influence the mech-

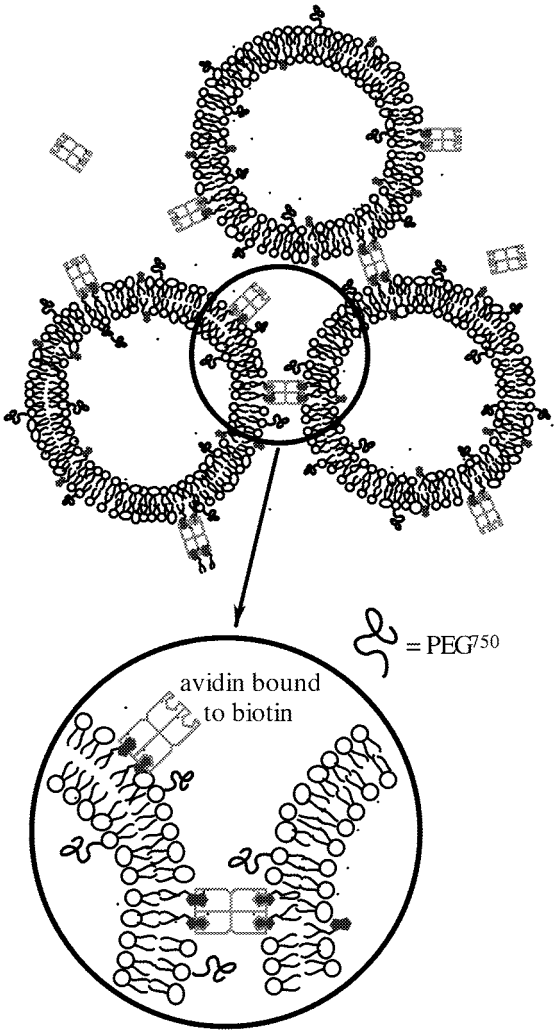


Figure 2. Schematic representation of the aggregation of biotinylated liposomes induced by the tetrameric streptavidin protein.

anical equilibrium established between the bilayers because it was not compressed in the contact zone but on the contrary PEG performed an important function by eliminating non-specific adhesion.

In a recent work (Sideratou *et al.*, 2002a) liposomes with recognizable groups were allowed to interact with dendrimers, which bear at their external surface complementary groups to liposomes. Thus, liposomes based on phosphatidylcholine and cholesterol and also incorporating dihexadecyl hydrogen phosphate as a recognizable lipid interacted with partially guanidinylated poly(propylene imine) dendrimers of the fourth and fifth generation as shown schematically in Figure 3.

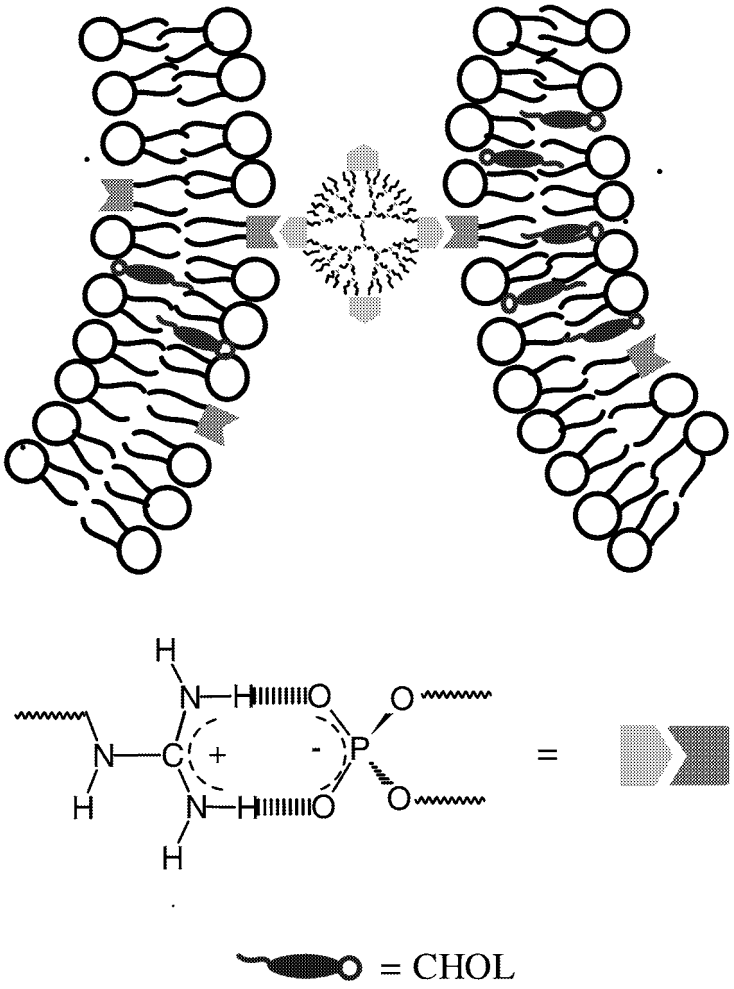


Figure 3. Schematic representation of the aggregation of phosphatidylcholine liposomes, containing various amounts of cholesterol (CHOL) and incorporating a recognizable moiety, induced by complementary dendrimers.

It should be noted that the diameter of the dendrimers employed is approximately the same with the thickness of the bilayer membrane (Sayed-Sweet *et al.*, 1997). The main results of the study are the following. (a) The higher generation dendrimeric derivative proved more effective in its interaction with liposomes. This behaviour was attributed to multivalent effects (Onda *et al.*, 1996), which are more pronounced for this dendrimeric derivative. Thus under an equal concentration of functional moieties the reactivity is favoured when the groups are located in a smaller number of particles. (b) The process is reversible since redispersion of the resulting aggregates was achieved by the addition of a phosphate buffer. (c) The liposomes were not disrupted during the collapse and redispersion processes as

assessed by the calcein fluorescence method, TEM and AFM (Sideratou *et al.*, 2002a).

The interaction of liposomes leading to the formation of tethered liposomes (bound liposomes preserving their size and shape) and/or large aggregates cannot however be generalized. Depending on the type of the interacting species transformation to other type of aggregates may occur. Thus by the interaction of dihexadecyl hydrogen phosphate liposomes with aqueous solutions of guanidinium cations ($C(NH_2)_3^+$) and its derivatives, the size of liposomes decreases and the aggregates are finally transformed into micelles at 1:1 molar concentration of the interacting moieties (Paleos *et al.*, 1999). In this case the guanidinium counterions are bound to the phosphate groups by combined electrostatic and hydrogen bonding forces resulting in an increase of the headgroup area. In this way and in analogy with simple ionic counterions, the known surfactant parameter requirement (Evans and Ninham, 1986), $\upsilon/\alpha\ell < 1/3$ is fulfilled for the formation of micelles where υ is the volume per hydrocarbon chain, or of the hydrophobic region of the surfactant, ℓ is an optimal hydrocarbon chain length related to the maximum extended length and α is the head group area.

3. Liposome-Liposome Interactions through Hydrogen Bonding

The introduction of complementary functional groups to a pair of liposomes, with the property of forming hydrogen bonds, can lead to the formation of large aggregates. During this process the following steps are involved (Cevc and Richardsen, 1999). (a) Adhesion in which the liposomes are simply conjoined but retain intact their inner compartments and (b) fusion in which the liposomes merge sharing a common inner compartment. In this second stage a sequence of processes occurs giving rise to the mixing of the aqueous content of liposomes with or without leakage or rupturing of the fused vesicles. Concerning the mechanism of membrane fusion (Cevc and Richardsen, 1999) two processes have been investigated, i.e. the conventional one involving the non-lamellar fusion and the second method proceeding through restructuring and ultimately merging of the membrane on a less ordered basis. It is supported that both mechanisms are energetically favorable at least for small-scale fusion. It has however to be noted that small liposomes, e.g. with diameters of about 45 nm comprising of less than 10,000 molecules, do not have sufficient number of amphiphiles to form a non-lamellar phase without disintegrating; these liposomes are however known to fuse extensively, apparently through membrane restructuring (Cevc and Richardsen, 1999). Taking into consideration the above mentioned associative mechanisms and processes such as the exchange of amphiphiles or the effect of liposomal size on the efficiency of their interaction the cited examples will be discussed.

In an early preliminary experiment (Paleos *et al.*, 1996) the amphiphiles 5,5-didodecylbarbituric acid, **1**, and 9-hexadecyladenine, **2**, were incorporated

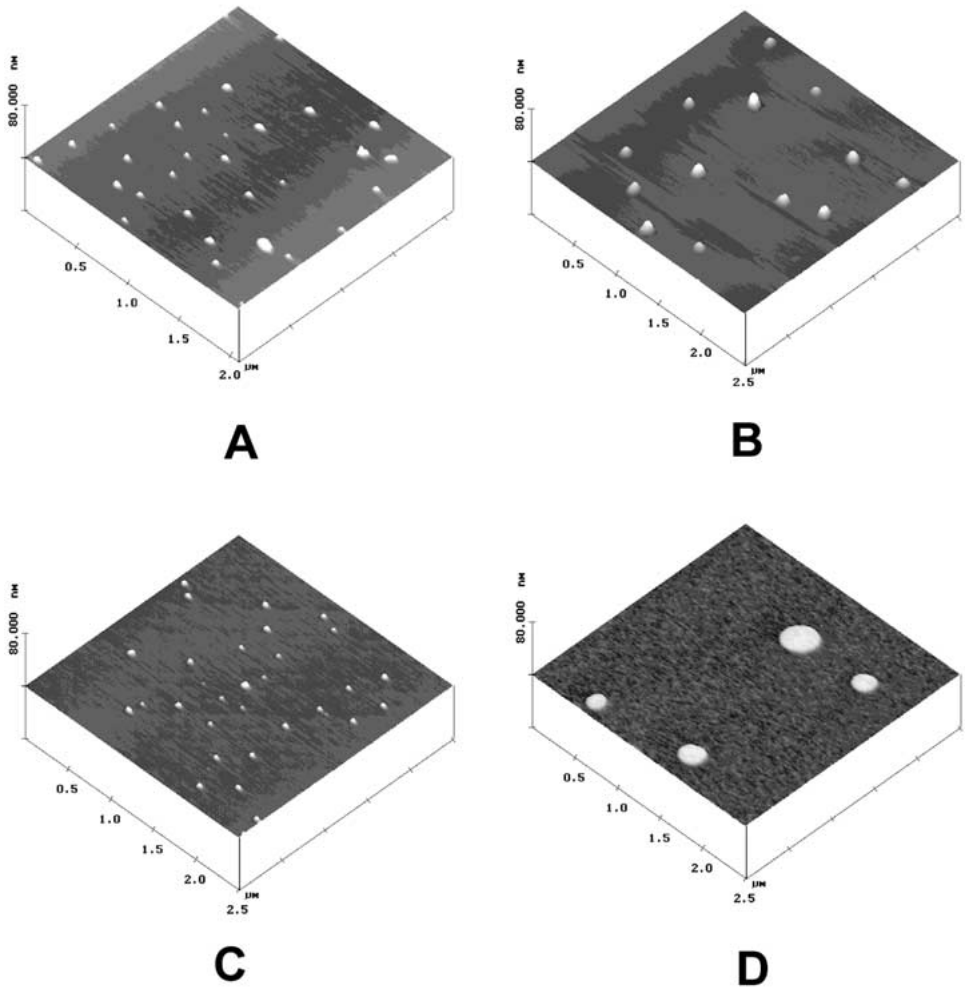
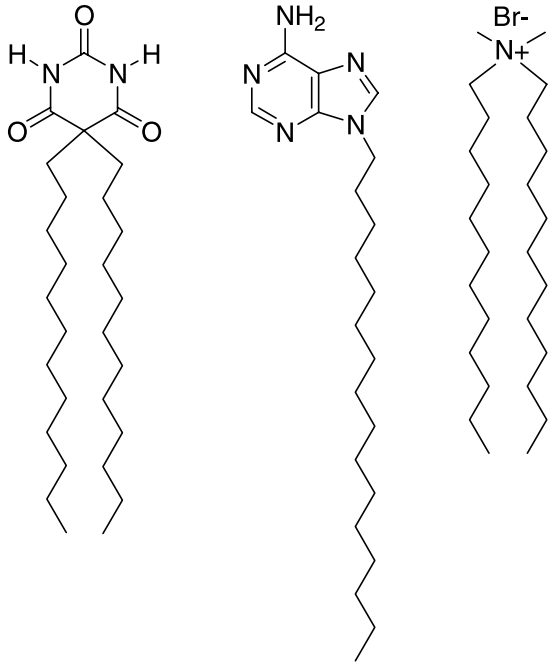


Figure 4. Images obtained with AFM microscopy of simple vesicles derived from **3** (A), mixed vesicles of **3** with **1** (B) or **2** (C) respectively, and vesicles (D) obtained after the interaction of B and C liposomes (Taken from Paleos *et al.*, 1996).

separately at an approximately 1/7 molar ratio to liposomes based on didodecyldimethylammonium bromide, **3**. Following liposomal interaction the aggregates obtained were larger than the original ones as revealed by Phase Contrast Optical and Atomic Force microscopies (Figure 4). It is interesting to note that these mixed molecular recognizable liposomes exhibit excellent stability and one has to add more than about 40% alcohol in order to be destructed.

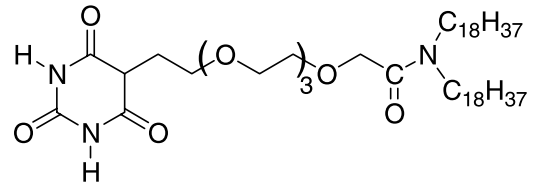
Extending the previous work, the same recognizable amphiphiles **1** and **2**, were now incorporated in liposomes based on neutral lipids consisting of hydrogen-



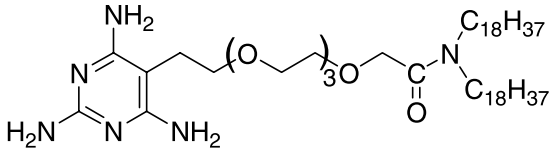
1 **2** **3**

ated soybean phosphatidylcholine (PC) and cholesterol (CHOL) in a PC:CHOL 2:1 molar ratio. Relatively high molar content of the recognizable molecules **1** or **2** (1:4 with respect to PC) was needed for quantitatively assessing liposomes recognition properties and obtaining measurable thermodynamic parameters. At these high concentrations of recognizable molecules incorporated in the PC and the PC-cholesterol based liposomes, the bilayer order may be severely affected and merited investigation. Thus, for the PC:CHOL unilamellar liposomes the high molar concentration of **1** and **2** relative to PC enforces a considerable degree of lateral heterogeneity in the bilayer and the lipids are not in the liquid-ordered phase but instead in the solid-ordered/liquid-ordered phase coexistence region (Sideratou *et al.*, 2002b).

Molecular recognition of liposomes becomes most effective at 1:1 molar ratio of the recognizable molecules as deduced from turbidity studies. In addition the incorporation of cholesterol in the bilayer of these liposomes enhanced their molecular recognition effectiveness, a result that will be further discussed with other types of liposomes. In addition AFM microscopy proved the interaction since liposomes with diameters 50 to 140 nm was transformed to aggregates with sizes between 300 to 450 nm. Furthermore, the initially collapsing and aggregating particles were relatively stable since no detectable disruption of the liposomes was observed as judged by calcein experiments.



4

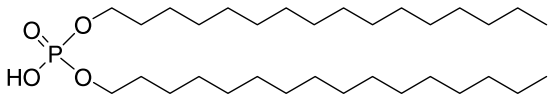


5

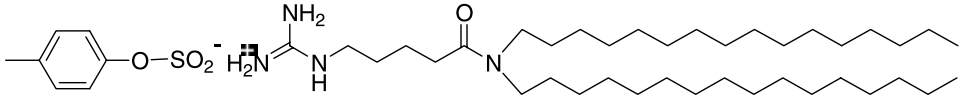
Quantitative investigation of the recognition of this pair of liposomes was performed with isothermal titration microcalorimetry (ITC). It has been found that one-to-one binding between adenine and barbituric acid in the lipid/water/lipid interface occurs. However at $T = 58\,^{\circ}\mathrm{C}$, above the main lipid phase transition, the situation is different and no liposomal binding can be detected. This is mainly due to the molecular disorder within the bilayer (liquid-disordered/liquid ordered phase coexistence) that limits the capacity of complementary moieties to bind, due to the weakening of the hydrogen bonds at these high temperatures.

The recognition of a complementary pair of liposomes based on lecithin and containing amphiphilic derivatives of barbituric acid, **4**, and triaminopyrimidine, **5**, respectively up to 10% molar ratio was thoroughly investigated by Lehn's group (Marchi-Artzner *et al*., 1997, 2001). In the first preliminary experiments (Marchi-Artzner *et al*., 1997), it was established that the complementary liposomes aggregate and fuse generating larger aggregates by employing freeze-fracture electron microscopy.

Recently the same complementary pair was employed and the consequence of the recognition features on aggregation, adhesion and fusion were extensively investigated (Marchi-Artzner *et al*., 1997) identifying the physicochemical parameters that participate in these processes. The main conclusions of this study are summarized in the following. (a) Rapid aggregation (in less than 30 s) occurs between the complementary liposomes, followed by lipid exchange (within 30 min after mixing). The lipid exchange, which takes place when the membranes are in contact, results either in fusion or, if fusion does not occur, to a redispersion of the liposomes within 17 hours. (b) The aggregation can be weakened by decreasing the ionic strength, through the addition of a soluble barbituric competitor or by decreasing the concentration of the recognizable amphiphiles. The effect of ionic strength underlines the basic role of electrostatic interaction in the initial



6



7

aggregation step. In addition the effect of the concentration of the recognizable amphiphiles supports the view that the recognizable system stabilizes the adhesion state. (c) The fusion process as observed by electron microscopy remained at a low level not resulting in an intermixing of the aqueous pools of the liposomes in significant proportion. (d) The size of the liposomes affects crucially the recognition phenomena. Thus aggregation was not observed when giant liposomes were employed. A rapid adhesion however occurs between complementary large and giant unilamellar liposomes.

Cholesterol, which is a basic constituent of cell membranes, affects their properties (Cooper, 1997) and therefore experiments with liposomes incorporating cholesterol may simulate its function in cell membranes and recognition. For this reason a pair of liposomes was prepared (Sideratou *et al.*, 2000) from PC and various amounts of cholesterol. These liposomes also incorporate di-n-hexadecyl hydrogen phosphate, **6**, and 1-(4-(dihexadecylcarbamoyl)butyl)guanidinium *p*-toluenesulfonate, **7**, as recognizable molecules, the guanidinium and phosphate groups of which interact strongly due to combined action of electrostatic forces and hydrogen bonding. The initially prepared small complementary liposomes, which were not possible to be observed by optical microscopy, became visible following their mixing and subsequent interaction. The initially formed aggregates, due to available reactive moieties further interact leading to even larger aggregates, which in certain cases encapsulate smaller liposomes as shown in Figure 5. The latter structures exhibit a kind of primitive compartmentalization reminiscent to that of biological cells.

Furthermore it was established that cholesterol incorporated in these liposomes significantly enhances their molecular recognition effectiveness. The molecular recognition enhancement, observed at cholesterol concentration levels of 10%–50% molar with respect to PC, can be attributed to the structural features of lipid-cholesterol bilayers. Membrane structure, at these levels of cholesterol con-

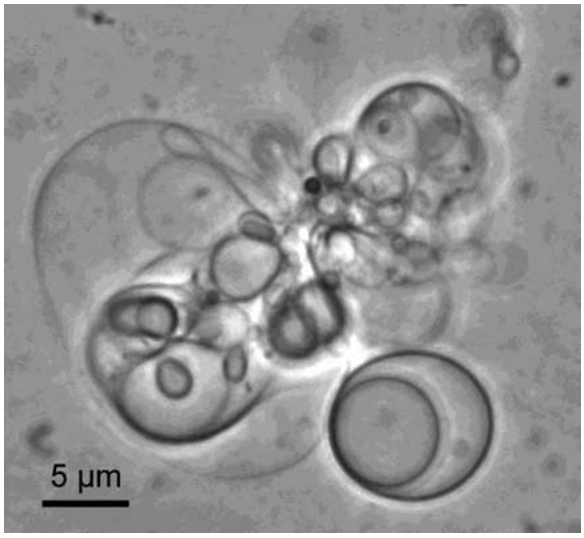
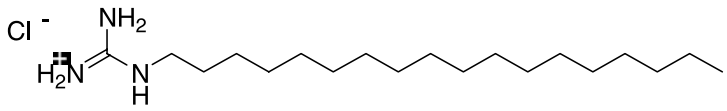


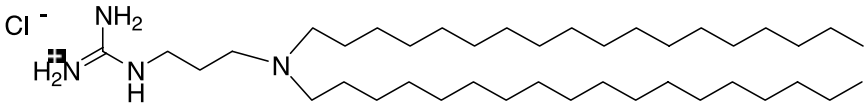
Figure 5. Phase-contrast optical microscopy images after mixing phosphatidylcholine-cholesterol based liposomes bearing the complementary guanidinium and phosphate moieties (Taken from Sideratou *et al*., 2000).

centration, contributes to a favourable exposure of the complementary groups of the lipids and the interaction is thus more efficient. As discussed (Sideratou *et al*., 2000) this is in agreement with the effect of cholesterol upon the molecular order of the lipid bilayer membrane according to a widely accepted phase diagram (Ipsen *et al*., 1987; Thewalt and Bloom, 1992; Vist and Davis, 1990; Trandum *et al*., 2000). According to this diagram, at the temperatures employed and with cholesterol in amounts higher than 25% molar concentration, the so-called liquid-ordered phase is obtained. The bilayer is in a fluid form from the point of view of lateral disorder and diffusion and therefore it affects the mobility of membrane-incorporated compounds. Since the incorporated recognizable lipids are at low concentrations (1:19 molar ratio), their presence do not appreciably perturb the molecular order of the PC-cholesterol bilayer and therefore the interacting moieties would most likely encounter the previously described organized environment. It is thus possible to tune the recognition efficiency of liposomes by changing the incorporated amounts of cholesterol in the liposomal bilayer. Based on these findings, model liposomal systems may be produced for exploring the role of cholesterol in molecular recognition in cell membranes.

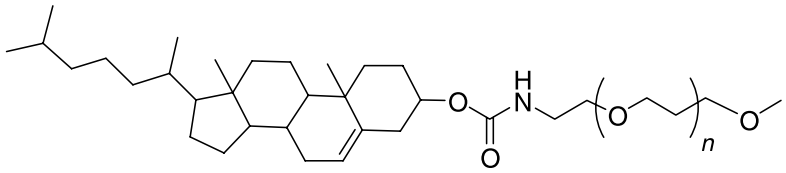
The inhibitory role of the polyethylene glycol (PEG) coating on molecular recognition was assessed by employing complementary liposomes based on PC and cholesterol (Pantos *et al*., 2002). These liposomes contained as recognizable lipids either octadecylguanidine hydrochloride, **8**, or N-[3-(N,N dioctadecylamino)propyl] guanidine hydrochloride, **9**, and di-n-hexadecyl hydrogen phosphate, **6**, respectively. PEG (molecular weight 5000) coating at the interface



8



9



10

of liposomes was introduced by incorporating varying amounts of PEGylated cholesterol, **10**. This derivative was anchored inside the membrane by its cholesteryl moiety. Segments of the interacting complementary particles are shown in Figure 6.

The interaction effectiveness between liposomes with the above mentioned recognizable pair of lipids was assessed by turbidimetry. A number of PEGylated cholesterol concentrations were tested in order to determine the optimum concentration at which both the interaction ability and the protection of liposomes is secured. As shown by turbidimetry (Figure 7), interaction between liposomes is significant at a 5% molar incorporation of PEGylated cholesterol, i.e., at a concentration in which effective protection is achieved in biological systems. Furthermore analogous results were obtained when **8** was replaced by **9**, i.e. a double chain amphiphile with the recognizable group attached through a spacer at the end of the lipophilic moiety. The interaction between liposomes incorporating **9** is rather more effective compared to liposomes incorporating **8**. Larger aggregates were obtained resulting in more turbid dispersions. This may be due to a better arrangement of

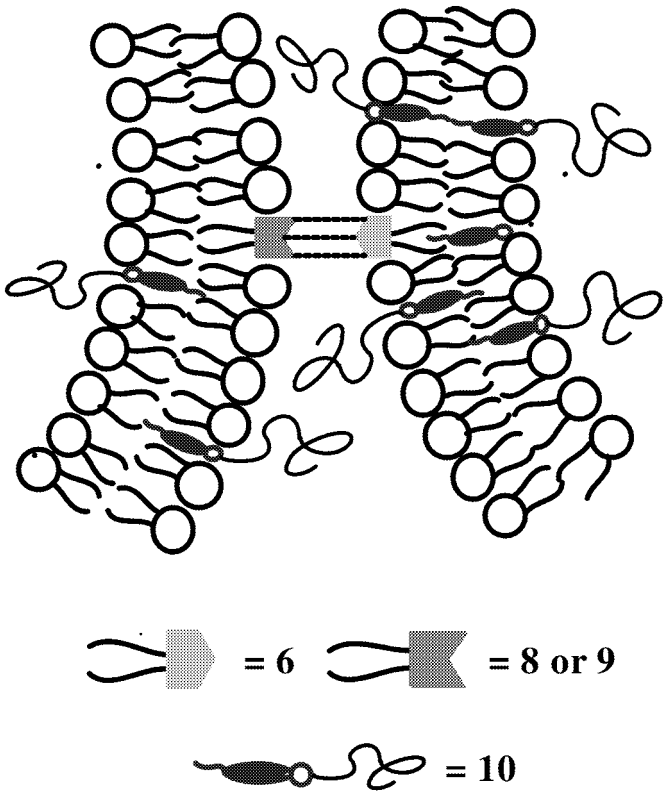


Figure 6. Schematic representation of molecular recognition between phosphatidylcholine-cholesterol based liposomes protected with PEG and bearing the complementary guanidinium and phosphate moieties respectively.

the double-chain amphiphile within the bilayer coupled with the presence of the spacer, which makes the recognizable group to protrude from the external surface of the bilayer. The same enhanced recognition was also observed for liposomes incorporating PEGylated cholesterol and **9** (Figure 7). In this case even with a 15% molar content of PEGylated cholesterol, interaction was effective while for liposomes containing **8** (Figure 7), 15% PEGylated derivative almost completely suppressed liposomes ability for interaction. Analogous results were also obtained microscopically since interactions occurred spontaneously and large aggregates were formed. These particles interact further giving rise to even larger aggregates, which in certain cases encapsulate smaller aggregates; in this manner compartmentalization is observed for the large aggregates. Association of the liposomes via the guanidinium-phosphate pair did not lead to disruption. As deduced from weak calcein fluorescence, self-quenching occurs since calcein remains entrapped at high concentrations in the interior of the liposomes (Komatsu and Okada, 1997; Osanai and Nakamura, 2000). Thus at these concentrations of the recognizable

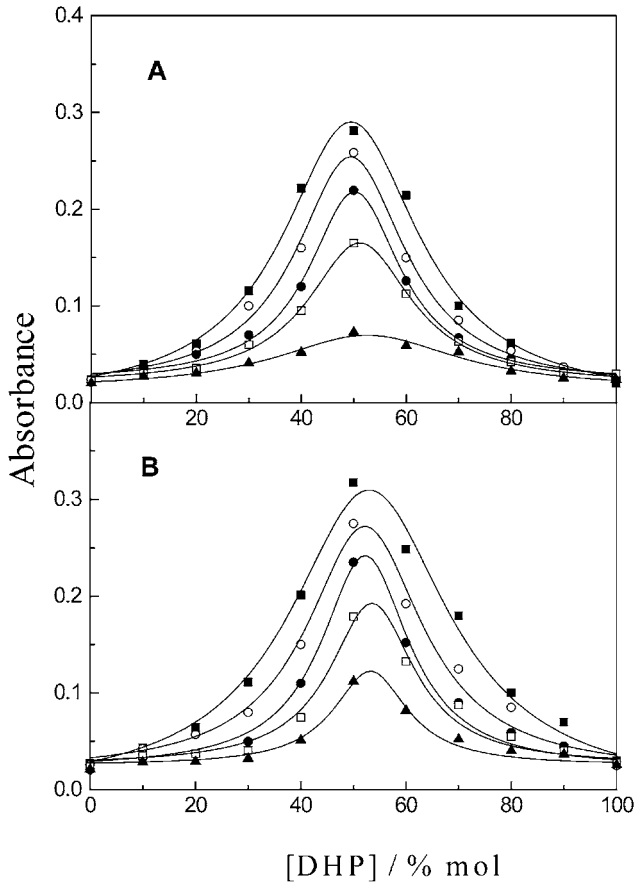
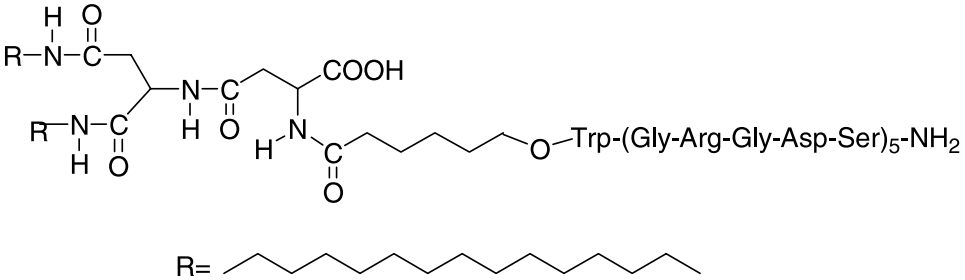


Figure 7. Turbidity change during interaction experiments between PC-CHOL-DHP and PC-CHOL-ODG (A) or PC-CHOL-DODG (B) liposomes. Both liposome dispersions incorporate PEGylated cholesterol at various concentrations. X axis represents the percent concentration of liposomes based on DHP in the aqueous dispersion: (■) 0%, (O) 1%, (●) 5%, (□) 8%, (▲) 15% PEG-Cholesterol (Taken from Pantos *et al*., 2002).

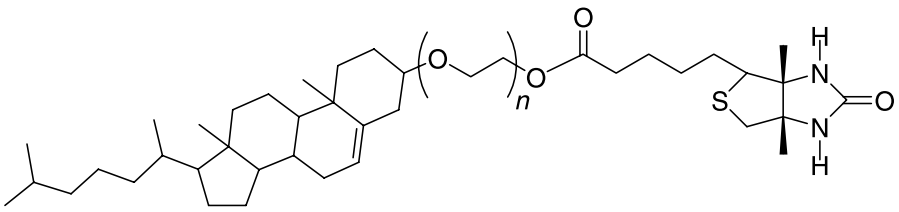
and protective functional moieties, a liposomal system was prepared that exhibited significant stability together with targeting.

4. Liposome-Cell Interactions through Complementary Moieties Located at their External Surface

In order to closer simulate the behaviour of biological cells, liposome-cell and cell-cell systems were studied, in the bilayer of which recognizable synthetic molecules were incorporated. The framework for these interactions (Lee *et al.*, 1992) has been set by employing two mammalian cell lines, i.e. CV1 and J774. It was finally



11



12

concluded that the rate of liposome uptake is not only controlled by the negative charge on the liposome surface but also by the specificity of the head group of the lipid.

On analogous manner the lipopeptide RGD-C4A2, **11**, was anchored with its lipophilic moieties in phospatidylcholine liposomes prepared by the well-established film method (New, 1990). These surface modified liposomes were found to bind to the membrane of NIH3H3l cells by intermolecular interactions (Yagi *et al*., 1999).

In this context, the last example although it involves relevant processes to the ones between liposomes it however refers to cell-cell interaction. The surfaces of these cells became recognizable through the incorporation of appropriate synthetic amphiphiles in their bilayer by incubation. In this case the aggregation of the cells was achieved by functionalized poly(ethyleneoxides), **12** (PEGs) (Meier, 2000) bearing at one end a hydrophobic cholesteryl group and at the other end a hydrophilic biotin group. These PEGs were anchored in the membrane of SubT1 cells (a human CD4 expressing T-lymphoblastoil cell line) via their cholesteryl group. The length of the PEG chain must be such as to protrude the glycocalyx of the cell in order to be accessible for interaction with the streptavidin added in the medium. Thus the molecular weight of PEG spacer chain was varied between 2000 and 35000 g mol^{-1}, i.e., its root-mean-square end-to-end distance being approximately

6 to 20 nm. It is interesting to note that this aggregation is reversed by the addition in the cell dispersion a large excess of free biotin. It is therefore reasonable to assume that the biotinylated polymer has a significantly lower binding affinity for streptavidin compared to free biotin. As claimed streptavidin aggregation is not only shared by lymphoblastoid cells but it can also occur with hepatocytes or bacteria.

5. Concluding Remarks and Future Prospects

The incorporation in the liposomal bilayer membrane of amphiphiles bearing polar groups, which are capable of forming hydrogen bond(s), induces their interaction with complementary molecules or liposomes. The supramolecular aggregates obtained usually exhibit more elaborated structures compared to their interacting counterparts. Complementarity of the functional groups and a favorable interplay (Yagi *et al*., 1999) of entropic and enthalpic effects are the driving forces for the formation of these aggregational-based biomaterials, which in certain cases exhibit multi-compartmental properties. Although the building of tissue-like materials is an ultimate goal of this research only the first steps towards this end have been done; intensification of the effort together with the design of ingenious experiments are required for accomplishing this goal. In addition, such studies contribute to further establishing the 'Lipid World' scenario (Segré *et al*., 2001; Ourisson and Nakatani, 1996; Lyubarev and Kurganov, 1997; Norris and Raine, 1998) proposed as an early evolutionary step in the emergence of cell life on earth. Lastly these investigations substantially contribute in tackling current research problems associated with liposomal drug delivery.

References

Ahlers, M., Müller, W., Reichert, A., Ringsdorf, H. and Venzmer, J.: 1990, Specific Interactions of Proteins with Functional Lipid Monolayers – Ways of Simulating Biomembrane Processes, *Angew. Chem. Int. Ed. Engl.* **29**, 1269–1285.

Ariga, K. and Kunitake, T.: 1998, Molecular Recognition at Air-Water and Related Interfaces: Complementary Hydrogen Bonding and Multisite Interaction, *Acc. Chem. Res.* **31**, 371–378 and references cited therein.

Berti, D., Franci, L., Baglioni, P. and Luisi, P. L.: 1997, Molecular Recognition in Monolayers. Complementary Base Pairing in Dioleoylphosphatidyl Derivatives of Adenosine, Uridine, and Cytidine, *Langmuir* **13**, 3438–3444.

Berti, D., Luisi, P. L. and Baglioni, P.: 2000, Molecular Recognition in Supramolecular Structures Formed by Phoshpatidylnucleosides-Based Amphiphiles, *Colloids Surfaces A: Physicochemical Engineering Aspects* **167**, 95–103.

Bradley, J.-C., Guedeau-Boudeville, M.-A., Jandeau, G. and Lehn, J.-M.: 1997, Toposomes Bearing Stable Perforations by the Action of an Electric Field on Partially Polymerized Giant Vesicles, *Langmuir* **13**, 2457–2462.

Cevc, G. and Richardsen, H.: 1999, Lipid Vesicles and Membrane Fusion, *Advanced Drug Delivery Reviews* **38**, 207–232.

Chiruvolu, S., Walker, S., Israelachvili, J., Schmitt, F.-J., Leckband, D. and Zasadzinski, J. A.: 1994, Higher Order Self-Assembly of Vesicles by Site-Specific Binding, *Science* **264**, 1753–1756.

Cooper, G. M.: 1997, *The Cell. A Molecular Approach*, ASM Press, Washington, D.C., 467 pp.

Decher, G., Ringsdorf, H., Venzmer, J., Bitter-Suermann, D. and Weisgerber, C.: 1990, Giant Liposomes as Model Membranes for Immunological Studies: Spontaneous Insertion of Purified Kl-antigen (Poly-alpha-2,8-NeuAc) of Escherichia Coli, *Biochim. Biophys. Acta* **1023**, 357–364.

Evans, D. F. and Ninham, B. W.: 1986, Molecular Forces in the Self-Organization of Amphiphiles, *J. Phys. Chem.* **90**, 226–234.

Freitag, S., Le Trong, I., Klumb, L. A., Chu, V., Chilkoti, A., Stayton, P. S. and Stenkamp, R. E.: 1999, X-Ray Crystallographic Studies of Streptavidin Mutants Binding to Biotin, *Biomolecular Engineering* **16**, 13–19.

Fyfe, M. C. T. and Stoddart, J. F.: 1997, Synthetic Supramolecular Chemistry, *Acc. Chem. Res.* **30**, 393–401.

Ipsen, J. H., Karlström, G., Mouritsen, O. G., Wennerström, H. and Zuckermann, M. J.: 1987, Phase Equilibria in the Phosphatidylcholine-Cholesterol System, *Biochim. Biophys. Acta* **905**, 162–172.

Käs, J. and Sackmann, E.: 1991, Shape Transitions and Shape Stability of Giant Phospholipid Vesicles in Pure Water Induced by Area-to-Volume Changes, *Biophys. J.* **60**, 825–844.

Kawasaki, T., Tokuhiro, M., Kimizuka, N. and Kunitake, T.: 2001, Hierarchical Self-Assembly of Chiral Complementary Hydrogen-Bond Networks in Water: Reconstitution of Supramolecular Membranes, *J. Am. Chem. Soc.* **123**, 6792–6800.

Komatsu, H. and Okada, S.: 1997, Effects of Ethanol on Permeability of Phosphatidylcholine/Cholesterol Mixed Liposomal Membanes, *Chem. Phys. Lipids* **85**, 67–74.

Koshland, D. E., Jr.: 1994, The Key-Lock Theory and the Induced Fit Theory, *Angew. Chem. Int. Ed. Engl.* **33**, 2375–2378.

Lee, K.-D., Hong, K. and Papahadjopoulos, D.: 1992, Recognition of Liposomes by Cells: in vitro Binding and Endocytosis Mediated by Specific Lipid Headgroups and Surface Charge Density, *Biochim. Biophys. Acta* **1103**, 185–197.

Lemieux, R. U.: 1996, How water Provides the Impetus for Molecular Recognition in Aqueous Solution, *Acc. Chem. Res.* **29**, 373- 380.

Lichtenthaler, F. W.: 1994, 100 Years Schlussel-Schloss-Prinzip-What Made Fischer, Emil use this Analogy, *Angew. Chem. Int. Ed. Engl.* **33**, 2364–2374.

Luisi, P. L., Walde, P. and Oberholzer, T.: 1999, Lipid Vesicles as Possible Intermediates in the Origin of Life, *Current Opinion in Colloid and Interface Science* **4**, 33–39.

Lyubarev. A. E. and Kurganov, B. I.: 1997, Origin of Biochemical Organization, *BioSystems* **42**, 103–110.

MacDonald, J. C. and Whitesides, G. M.: 1994, Solid-State Structures of Hydrogen-Bonded Tapes Based on Cyclic Secondary Diamides, *Chem. Rev.* **94**, 2383–2420.

Mammen, M., Choi, S.-K. and Whitesides, G. M.: 1998, Polyvalent Interactions in Biological Systems: Implications for Design and Use of Multivalent Ligands and Inhibitors, *Angew. Chem. Int. Ed. Engl.* **37**, 2754–2794.

Marchi-Artzner, V., Jullien, L., Gulik-Krzywicki, T. and Lehn, J.-M.: 1997, Molecular Recognition Induced Aggregation and Fusion between Vesicles Containing Lipids Bearing Complementary Hydrogen Bonding Head-Groups, *Chem. Commun.*, 117–118.

Marchi-Artzner, V., Gulik-Krzywicki, T., Guedeau-Boudeville, M.-A., Gosse, C., Sanderson, J. M., Dedieu, J.-C. and Lehn, J.-M.: 2001, Selective Adhesion, Lipid Exchange and Membrane-Fusion Processes between Vesicles of Various Sizes Bearing Complementary Molecular Recognition Groups, *CHEMPHYSCHEM* **2**, 367–376.

Meier, W.: 2000, Reversible Cell Aggregation Induced by Specific Ligand-Receptor Coupling, *Langmuir* **16**, 1457–1459.

Menger, F. M. and Lee, S. J.: 1995, Induced Morphological Changes in Synthetic Giant Vesicles: Growth, Fusion, Undulation, Excretion, Wounding, and Healing, *Langmuir* **11**, 3685–3690.

Miller, A. D.: 2002, Order for Free: Molecular Diversity and Complexity Promote Self-Organization, *CHEMBIOCHEM* **3**, 45–46.

New, R. R. C.: 1990 *Liposomes, a Practical Approach*, IRL Press, Oxford.

Noppl-Simson, D. A. and Needham, D.: 1996, Avidin-Biotin Interactions at Vesicles Surfaces: Adsorption and Binding, Cross-Bridge Formation, and Lateral Interactions, *Biophys. J.* **70**,1391–1401.

Norris, V. and Raine, D. J.: 1998, A Fission-Fusion Origin for Life, *Orig. Life Evol. Biosphere* **28**, 523–537.

Onda, M., Yoshihara, K., Koyano, H., Ariga, K. and Kunitake, T.: 1996, Molecular Recognition of Nucleosides by the Guanidinium Unit at the Surface of Aqueous Micelles and Bilayers. A Comparison of Microscopic and Macroscopic Interfaces, *J. Am. Chem. Soc.* **118**, 8524–8530.

Osanai, S. and Nakamura, K.: 2000, Effects of Complexation between Liposomes and Poly(malic acid) on Aggregation and Leakage Behaviour, *Biomaterials* **21**, 867–876.

Ourisson, G. and Nakatani, Y.: 1996, Can the Molecular Origin of Life be Studied Seriously?, *C. R. Acad. Sci. Paris, Serie II* **322**, 323–334.

Paleos, C. M., Sideratou, Z. and Tsiourvas, D.: 1996, Mixed Vesicles of Didodecyldimethyl Ammonium Bromide with Recognizable Moieties at the Interface, *J. Phys. Chem.* **100**, 13898–13900.

Paleos, C. M. and Tsiourvas, D.: 1997, Molecular Recognition of Organized Assemblies via Hydrogen Bonding in Aqueous Media, *Advanced Materials* **9**, 695–710 and references cited therein.

Paleos, C. M., Tsiourvas, D. and Kardassi, D.: 1999, Transformation of Dihexadecyl Phosphate Vesicles to Micelles Using Guanidinium-Type Counterions, *Langmuir* **15**, 282–284.

Paleos, C. M., Sideratou, Z. and Tsiourvas, D.: 2001, Molecular Recognition of Complementary Liposomes in Modeling Cell-Cell Recognition, *CHEMBIOCHEM* **2**, 305–310.

Pantos, A., Sideratou, Z. and Paleos, C. M.: 2002, Complementary Liposomes Based on Phosphatidylcholine: Interaction Effectiveness vs Prospective Coating, *J. Colloid Interface Sci.* **253**, 435–442.

Prins, J. L., Reinhoudt, D. N. and Timmerman, P.: 2001, Noncovalent Synthesis Using Hydrogen Bonding, *Angew. Chem. Int. Ed. Engl.* **40**, 2382–2426.

Rebek, J., Jr.: 1996, Molecular Recognition and Assembly, *Acta Chem. Scand.* **50**, 707–716.

Rosano, C., Arosio, P. and Bolognesi, M.: 1999, The X-Ray Three-Dimensional Structure of Avidin, *Biomolecular Engineering* **16**, 5–12.

Sayed-Sweet, Y., Hedstrand, D. M., Spinder, R. and Tomalia, D. A.: 1997, Hydrophobically Modified Poly(amidoamine) (PAMAM) Dendrimers: Their Properties at the Air-Water Interface and Use as Nanoscopic Container Molecules, *J. Mat. Chem.* **7**, 1199–1205.

Schief, W. R., Edwards, T., Frey, W., Koppenol, S., Stayton, P. S. and Vogel, V.: 1999, Two-Dimensional Crystallization of Streptavidin: In Pursuit of the Molecular Origins of Structure, Morphology, and Thermodynamics, *Biomolecular Engineering* **16**, 29–38.

Segré, D., Ben-Eli, D., Deamer, D. W. and Lancet, D.: 2001, The Lipid World, *Orig. Life Evol. Biosphere* **31**, 119–145.

Sideratou, Z., Tsiourvas, D., Paleos, C. M., Tsortos, A. and Nounesis, G.: 2000, Molecular Recognition of Complementary Liposomes: The Enhancing Role of Cholesterol, *Langmuir* **16**, 9186–9191.

Sideratou, Z., Foundis, J., Tsiourvas, D., Nezis, I. P., Papadimas, G. and Paleos, C. M.: 2002a, A Novel Dendrimeric 'Glue' for Adhesion of Phospotidyl Choline Based Liposomes, L*angmuir* **18**, 5036–5039.

Sideratou, Z., Tsiourvas, D., Paleos, C. M., Tsortos, A., Pyrpassopoulos, S. and Nounesis, G.: 2002b, Interaction of Phosphatidylcholine Based Liposomes Functionalized at the Interface with Adenine and Barbituric Acid Moieties, *Langmuir* **18**, 829–835.

Thewalt, L. J. and Bloom, M.: 1992, Phosphatidylcholine-Cholesterol Phase-Diagrams, *Biophys. J.* **63**, 1176–1181.

Trandum, C., Westh, P., Jørgensen, K. and Mouritsen, O. G.: 2000, A Thermodynamic Study of the Effects of Cholesterol on the Interaction between Liposomes and Ethanol, *Biophys. J.* **78**, 2486–2492.

Vist, M. R. and Davis, J. H.: 1990, Phase Equilibria of Cholesterol/Dipalmitoylphosphatidylcholine Mixtures: ^{2}H Nuclear Magnetic Resonance and Differential Scanning Calorimetry, *Biochemistry* **29**, 451–464.

Whitesides, G. M., Simanek, E. E., Mathias, J. P., Seto, C. T., Chin, D., Mammen, M. and Gordon, D. M.: 1995, Noncovalent Synthesis: Using Physical-Organic Chemistry to Make Aggregates, *Acc. Chem. Res.* **28**, 37–44.

Yagi, N., Ogawa, Y., Kodaka, M., Okada, T., Tomohiro, T., Konakahara, T. and Okuno, H.: 1999, A Surface-Modified Functional Liposome Capable of Binding to Cell Membranes, *Chem. Commun.*, 1687–1688.