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Review

Structural aspects of ganglioside-containing membranes

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ABSTRACT

The demand for understanding the physical role of gangliosides in membranes is pressing, due to the high number of diverse and crucial biological functions in which they are involved, needing a unifying thread. To this purpose, model systems including gangliosides have been subject of extensive structural studies. Although showing different levels of complication, all models share the need for simplicity, in order to allow for physico-chemical clarity, so they keep far from the extreme complexity of the true biological systems. Nonetheless, as widely agreed, they provide a basic hint on the structural contribution specific molecules can pay to the complex aggregate. This topic we address in the present review. Gangliosides are likely to play their physical role through metamorphism, cooperativity and demixing, that is, they tend to segregate and identify regions where they can dictate and modulate the geometry and the topology of the structure, and its mechanical properties. Strong three-dimensional organisation and cooperativity are exploited to scale up the local arrangement hierarchically from the nano- to the mesoscale, influencing the overall morphology of the structure.

Contents

1. Introduction

Gangliosides belong to the class of biological amphiphiles associated to lipid-driven membrane domains, that is, immiscible finite lipid nanostructures within a hosting lipid matrix. Their huge hindrance and their peculiar geometry would assign them a prominent role in defining the properties of the nanostructure. The long-aged claim for a decisive involvement of gangliosides in both structural and functional properties of the microdomains has stimulated a great deal of experimental work along nearly 3 decades. Several experimental techniques have been applied, either focused on the thermodynamics, like calorimetry or lateral surface pressure [1–7], and on the structure, like radiation spectroscopy or microscopy and conductometry [8–23], and on interparticle interactions, like pressure-distance measurements or X-ray and neutron spectroscopy [24–27], on pure ganglioside solutions or on mixtures with other lipid components. They have been periodically revisited [28–32] every time new emerging ideas were bringing about new promising hints. Nonetheless, at the state of art, they still seem rather to profit from the structural features imposed by other microdomain components, like cholesterol [33], rather than playing a fundamental role in the domain organisation.

Curvature, metamorphism, cooperativity and demixing are the physical properties that have emerged as contributions provided by gangliosides to the aggregates where they are embedded. They play with each other resulting in complex structural and dynamic properties. Some aspects are shared with other amphiphiles, like those due to the presence of charged groups, but give rise to peculiar behaviours

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through coupling with specific properties. Some aspects become evident only when mixed with other molecules, or if the "third dimension" is more than thickness, or when asymmetric distribution is forced, like in real membranes.

The structural story of gangliosides can be written by following two main representatives, GM1 and GM3, sketched in Fig. 1 in comparison with the phospholipid DMPC. GM1 is used as the ganglioside paradigm. It displays the characteristic features identifying gangliosides towards other membrane amphiphiles, and, not least, widely employed for its accessibility. GM3 is a borderline-ganglioside, that has increasingly attracted attention in connection to its central role in tumour progression. Its ability to modulate the geometry and the mechanical properties of the aggregated structure is invoked to explain the mechanism for function [34,35].

2. Curvature

Curvature is the characterising feature of most ganglioside aggregates in dilute solution [36], as compared to the flatness of phospholipids membranes. This is the nanoscale result of the geometry of the ganglioside molecules, following strongly sterical local arrangement. The hindrance of the headgroup in this class of glycolipids is huge, facing the extended double tail ceramide moiety, and paying a strong contribution to the hydrophobic-hydrophilic balance. According to the well known scheme of Israelachvili [37] gangliosides have a packing parameter less than, and close to, one half, giving rise to aggregates with non negative average curvature, as sketched in Fig. 2. Any variation, even small, in the headgroup hindrance produces amplified effects on the aggregate size. Consistent modifications to the aggregate curvature, for example, are associated to the lactone derivatives of gangliosides [38]. Correspondingly, differences are detected in the effect played by ganglioside lactones on the packing with phospholipids in mixed monolayers, passing from condensing to expanding with the extent of lactonisation. This effect, once translated in a real membrane, has been indicated as putatively involved in an amplified structural response to a minor modification that does not involve metabolic cycling [39].

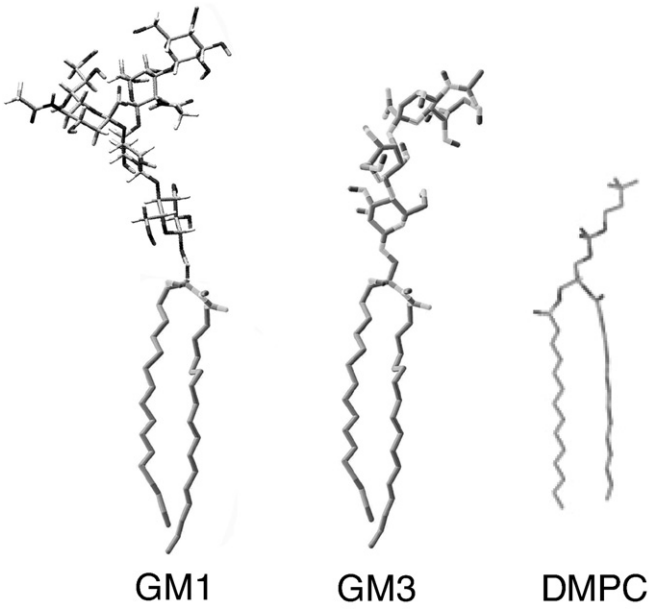

Fig. 1. Molecular structures. Stick representation of GM1 and GM3 gangliosides. The structure of DMPC is reported for comparison. GM1: β-Gal-(1-3)-β-GalNAc-(1-4)-[α-Neu5Ac-(2-3)]-β-Gal-(1-4)-β-Glc-(1-1)-Cer; GM3: α-Neu5Ac-(2-3)-β-Gal-(1-4)-β-Glc-(1-1)-Cer; DMPC:1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine.

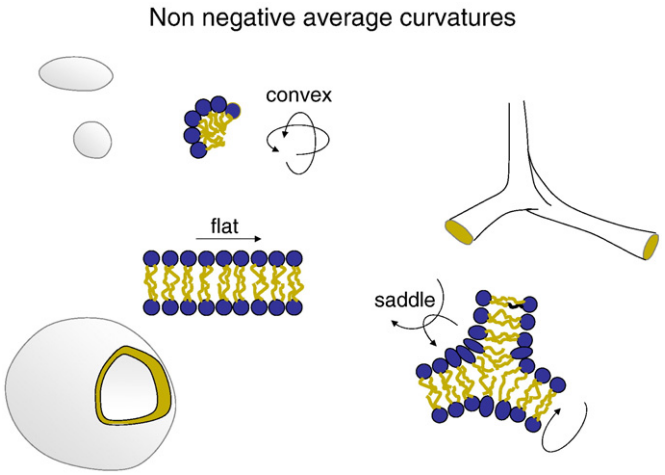

Fig. 2. Curvature and metamorphism. Several forms of ganglioside aggregates are sketched to illustrate the possibility offered by multiple headgroup packing, to obtain different local curvatures on the surface, from highly positive, in micelles, down to zero average curvature of the saddle geometry (positive at least along one line) in the branched aggregate.

On the other hand, the curvature of ganglioside aggregates is strictly mastered, as evidenced by their unusual behaviour as a function of both the concentration [26] and the ionic strength of the solution [40]. In fact, although being charged and then subjected to intra-aggregate and inter-aggregate electrostatic interactions, usually driving curvature to decrease when side–side interactions fade or front–front interactions grow, ganglioside micelles keep their geometry until a collective event takes place, turning curvature to a different defined state [41].

As usual in aggregates of ionic amphiphiles, counterion distribution around charged surfaces provides them with only a partial net, or effective, charge [42]. The effective charge of GM1 micelles, for example, is of the order of 1:6 as compared to the structural charge [40]. Anyway, ionic dissociation does not seem to play a prominent role in ganglioside packing on the intra-aggregate scale, as compared to steric hindrance. On the other side, screening of the Coulomb potential is easily attained, on the inter-aggregate scale, by added electrolytes. Nonetheless, ionic dissociation could become important on a local scale within a membrane surface, paying a definite negative sign to finite regions containing gangliosides, contributing to surface templating [43] or participating to the dynamics of cell adhesion clusters [44].

Curvature is a way to escape from two-dimensionality, that is, a way to protrude on the nanoscale from a flat membrane, or to pull or torque aggregates to complex tridimensional mesostructures that are indeed encountered, at least locally, in the vast morphology of real membranes [45–49].

The complex phase diagram of GM1 is dictated by the irreducible non-negative-curvature requirement. Of course, the packing of GM1 molecules has to change as concentration is raised, but only direct phases are encountered. Among them, an unusually wide region is found where bicontinuous structures are formed, of the diamond and gyroid type (saddle-shaped surfaces). There, ganglioside assemblies stand zero average-curvature while assuming negative Gaussian-curvature, that is, they find a collective headgroup arrangement allowing for a positive bending at least along one direction [50]. This kind of asymmetry in curvature (negative Gaussian curvature) is similar to that found in the neck regions joining a flat portion of a cell membrane and a protruding filament or flask or an indented caveola. A similar local deformation should occur on vesicle budding from a parent membrane or on vesicle merging with a target one, like in the biological processing of matter from the Endoplasmic Reticulum and through the Golgi apparatus. In this region of the cell, cubic

membranes, that is, a complex system of membranes assuming a wrapped ordered geometry, have been observed [45]. Gangliosides, there biosynthesised, are then likely to concur to the tuning of membrane deformation and folding.

Curvature is also thought as a driving agent to segregation on membranes, contributing to the definition of nanoscale environments where lipids with similar anomalous curvature requirements, hardly spread in flatlands, could merge to find an optimal packing, meanwhile dictating the geometry of the membrane region. This long lived track, often put forward among the motivations for investigating ganglioside micellar solutions, has been recently revisited to recall its interplay with membrane dynamics [51]. Curvature on the mesoscale has also been proposed as a structural motif on the membrane, exploited for curvature-based recognition of large macromolecules [52].

We underline here that the structural properties of the individual molecules are of course contributing to the overall mechanical characteristics of a complex mixed auto-aggregating system while facing, mixing, averaging, and compensating with other molecules, of the same and of different types. From a simple "geometrical" standpoint, a puzzle can be composed where a combination of suitable molecules of different packing shapes results in a flat overall aggregate portion [53].

Nonetheless, the physical properties of the heterogeneous ensemble are the outcome of this complex interplay that may result in overall mechanical features like, for example, higher local deformability or structural compactness, identifying a multi-molecular environment as a single entity, eventually travelling along the membrane, or across [54]. Besides shape-averaging, GM1 and GM3 have been found to locate in different regions of the apical domain of epithelial cells, characterised by different curvatures [55].

3. Metamorphism

Of course, all amphiphiles undergo modifications of their packing geometry while crossing the phase diagram. On increasing concentration, the complex interplay with similar molecules and water forces a decrease in the aggregate curvature passing from direct to flat, to reverse phases [37]. A major structural feature of ganglioside aggregates is that their shape can be modulated at constant concentration. Individual molecules can display multiple space filling geometries [56–59], collectively exploited in aggregates and amplified in nanoscale metamorphism. This feature is particularly intriguing in the case of GM3, having quite an equilibrated hydrophilic-hydrophobic balance, with a preferred molecular packing parameter very close to one half, in the region where discrimination between the world of flat and convex shapes occurs. In dilute solution, GM3, besides spontaneous unilamellar vesicles, packs into coexisting structures that are non-homogeneous in the surface curvature over the single aggregate, such as finite portions of flat lamellas, or small lamellar fragments [10–12,60–62]. These structures, characterised by the demand of compensating the edge exposure, are stabilised by increasing the surface coverage per headgroup, locally providing the required curvature contribution. Spontaneous vesiculation itself is a rare event in mono-component systems, dealing with the possibility of curving the inner and the outer leaflet of the membrane surface in an asymmetric way without energy cost. This is usually obtained by mixing different components, non homogeneously populating the two leaflets [63,64], providing the wrapping membrane with a spontaneous curvature. In the case of GM3, vesicle closure is played on low bending energy, a feature that could be translated in complex membranes by promoting collective deformations from the flat geometry. Addition of GM1 to GM3 leads to a decrease of lamellar aggregates [65], stabilising mixed vesicles by spontaneous curvature.

4. Cooperativity

Amphiphile molecular metamorphism is translated to the nano- and mesoscale through cooperativity, defining ensemble properties like core fluidity, bending rigidity, and curvature. For lipids aggregating in lamellar-type assemblies, the really stringent tie to the policy of structural arrangement and rearrangement, in response to variations in the thermodynamic conditions, usually comes from the hydrophobic tails. Their cooperative behaviour gives rise to the variety of confined phases, namely L_{α}, L_{β}, $L_{\beta'}$, $P_{\beta'}$ according to the conformation assumed by their chains within the hydrophobic region, even in the disperse regime, that is in the absence of a widespread order [66]. In ganglioside aggregates, cooperative rearrangement takes place also in the hydrophilic region [67,68], corresponding to different headgroup packing on the surface, resulting in different surface coverage ability. In fact, a wider packing geometry can be induced collectively either by heating or by increasing concentration in intermediate ranges [26], opposite to the general rule that curvature reduces as concentration increases [37], suggesting a nontrivial implication of solvent water structuring in the headgroup rearrangement. In this respect, a molecular dynamics simulation [69], focused on the motion of water in the presence of GM3 ganglioside bilayers, shows that water molecules initially close to the GM3 surface diffuse about an order of magnitude less than those in the bulk, reflecting the strong interaction between water molecules and the GM3 sugar groups.

Moreover, the surface collective rearrangement causes a response in the hydrophobic core of the aggregate [70], that is, headgroup and chains conformations of gangliosides are strongly coupled. If translated into a membrane, a ganglioside patch could then constitute a bistable device that could be activated, for example, by an approaching body altering the solvent structure. Headgroup rearrangement could constitute a switchable coupling junction between inside and outside structures, along with the transduction function commonly assigned to gangliosides. The ability to switch cooperatively between different structures is maintained till a 1:3 surface dilution with a spacer molecule bearing a phosphocholine headgroup [71]. This ratio is significant, as it constitutes the threshold for a topological transition.

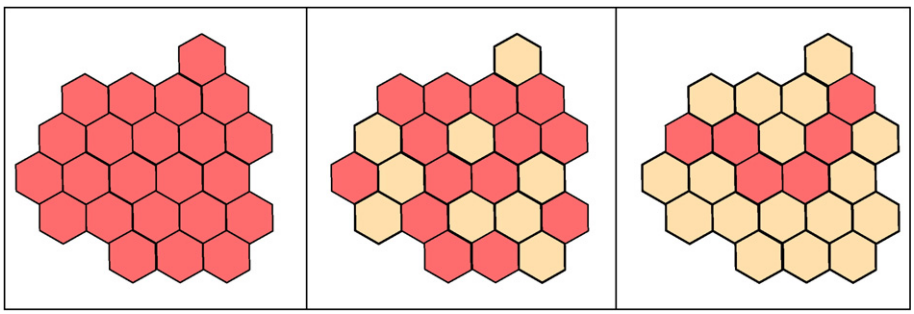

Fig. 3. Cooperativity. On membrane surfaces, the ability of gangliosides (grey hexagons) to switch cooperatively between different structures is maintained upon dilution with a spacer molecule (white hexagon), till a 1:3 ratio of ganglioside over spacers. Cooperating gangliosides keep in contact along a step-by-step walk.

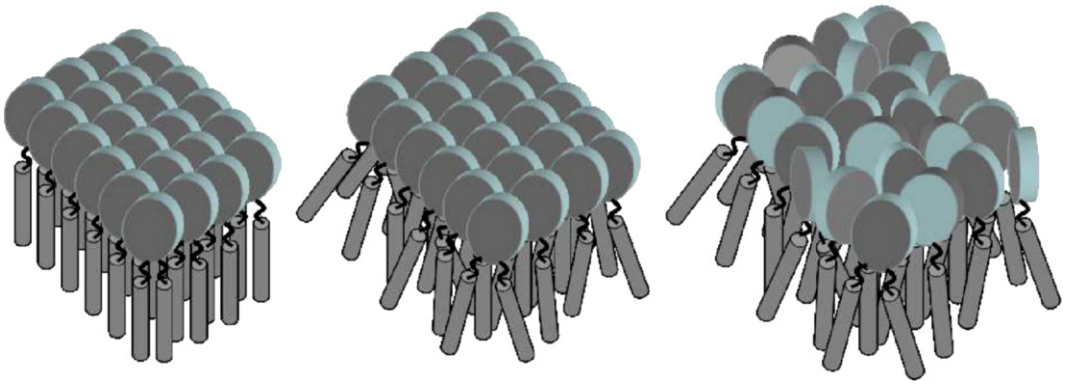

Fig. 4. GM3 phases. From left to right the solid ordered, solid disordered and liquid disordered phases of GM3 are sketched in liquid-crystal terms. Headgroups and tails are represented by discs and cylinders, respectively.

At 1:3 surface number density the molecules of the minority compound still can keep in contact with each other, see Fig. 3, capable of forcing the packing of a similar molecule. It is interesting to notice that the same ratio has been found to be the limiting value for the condensing effect to take place in mixed DPPC/GM1 monolayers and induce ordering [72]. Besides topological considerations, the fact that collective structural rearrangement is preserved until important surface dilution allows to assume this behaviour as exploitable in ganglioside-enriched domains in real membranes, where ganglioside are diluted among other components.

The interplay between headgroup packing and chain order gives rise to a major result in GM3 assemblies, showing a degree of structural cooperativity feebler in the region of the lipid chains and stronger in the region of the headgroups, and resulting in an intermediate phase where molten chains are held by structured headgroups. The existence of a strong degree of order on the molecular scale has been proposed to be due to a highly constrained and symmetric disposition of the trisaccharide charged headgroups of GM3 at the bilayer surface, occurring in a dispersed lipid assembly, in normal pressure conditions [73,74]. This structure can be assimilated to one of the various intermediate smectic phases, see Fig. 4, populating the liquid crystal world, originated by discotic-calamitic mesogens [75,76]. Pronounced ordering of GM3 headgroups has also been suggested by testing molecular dynamics simulation on NMR in bicelles [56].

The ability to draw ordered motifs on the surface of a hosting membrane is likely to constitute a major contribution of gangliosides to promoting morphological rearrangement of adhering macromolecules via structural templating, and to be at the basis of their role in amyloid induction [43].

5. Demixing

At present, there is a flourishing of theoretical papers addressing the topic of segregation, identifying the general rules governing the formation of membrane domains and with regards to extension and shape, with predictive potential. The problem can be approached from a general point of view [77] without invoking particular interactions, but moving in the same framework that describes micellization [78], as micellization itself is a demixing process played on the nanoscale, rather than on the macroscopic scale, giving rise to domains (the micelles) of condensed matter (a rich phase) dispersed in a dilute aqueous phase. In the case of demixing within a membrane, pictorially sketched in Fig. 5 (centre), a more subtle game is played, involving solubility (amphiphilicity) mismatch between admixed molecules. While usually mainly mastered in the hydrophobic core, also stringent hydrophilic structural requirements are brought about by the presence of gangliosides, being able even to demix from each other on the nanometric scale of a mixed micelle [79]. In addition, the pronounced extension of the ganglioside headgroups enlarges the mixing/demixing playground from a pseudo-surface to a true-thick layer, involving three-dimensional space filling. An example of mixing and demixing within a thick surface is sketched in Fig. 5 (right). Mixing can produce a condensing effect. This feature is reflected, for example, in the surface condensing effect played by gangliosides when intimately mixing with other lipids, as observed in mixed

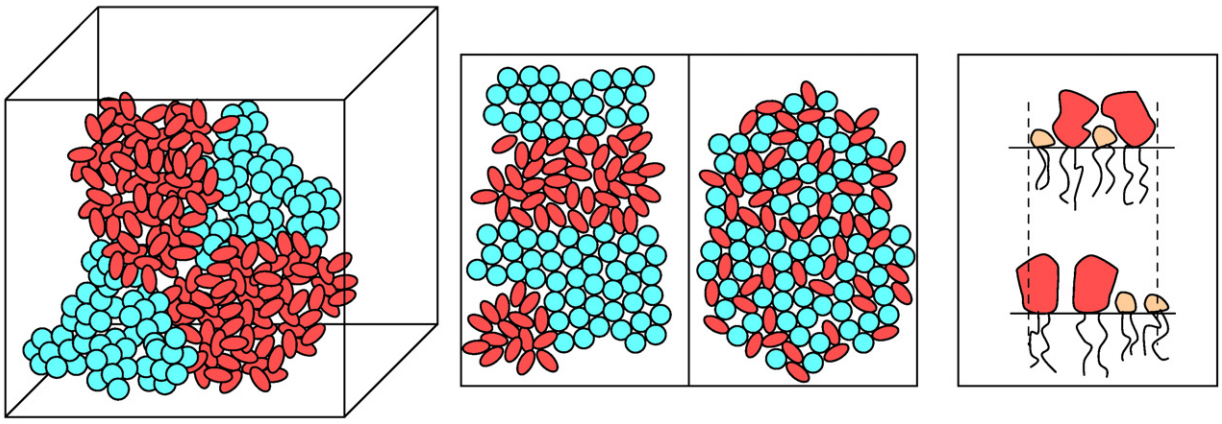

Fig. 5. Demixing. (Left) 3D demixing of different particles giving rise to bulk phase separation. (Centre) 2D top view of surface covering by a mixture of two different particles: phase separated and mixed configurations. (Right) Side view of mixing and demixing within a thick surface. Mixing can produce a condensing effect.

monolayers [39,72,80]. On the other hand, demixing played on headgroup interactions can drive to most exotic nanoscale aggregates, like the icosahedra formed by catanionic surfactant systems, looking like hard rather than soft matter [81,82].

Cluster formation and stability is played with mismatches, depicted as local, like molecular splay or tilt, or collective, like line tension or bending energy difference [83,84]. All of these parameters are likely to be strongly influenced by the presence of gangliosides. In particular, line tension along the domain edge can be relieved by monolayer bending, or molecular rearrangement. Recently, also the topic of the dynamics of domain formation has been addressed by simulation techniques, classifying admixed different molecules according to their reorientational dynamics [85]. This is an interesting aspect in the case of gangliosides, where both headgroups and tails can influence the dynamics of the environment, due to strongly organised surface packing. An intriguing aspect of ganglioside location on membranes is that they are hosted in domains identified by liquid-ordered arrangement, mainly attributed to the presence of cholesterol, while being themselves able to establish a solid-disordered structure. On the other hand, cholesterol prefers to interact with sphingolipids different from gangliosides [3,86]. This suggests an interesting structural role for the cholesterol/ganglioside pair in the setup of domain superstructuring in real membranes, through tunable fencing of "easily-walkable" cluster subregions. Anyway, gangliosides are able to demix from other lipids also in the absence of cholesterol [87], as well as the liquid ordered phase can be formed in mixed systems without cholesterol [88].

Recently, chirality has been considered as a factor determining the extension of domains, and their shape, that are known to assume non-flat morphologies, giving rise to caveolae or budding or flasky bodies protruding from the membrane or digging into it [89]. This is again a putative contribution of gangliosides to the membrane structure and morphology. In fact, tube casting from the surface is induced by addition of GM3, and other gangliosides, to phospholipids membranes [90], while chirality-imprinted structures are formed by glycosphingolipids [91].

6. Asymmetry

Gangliosides distribution across the real membranes is strongly asymmetrical. This situation is far from the trivial uneven distribution that is of course easily obtained in mixed artificial vesicles, providing them with a spontaneous curvature [63]. Gangliosides in real membranes are forced to reside only on the outer layer. This fact has been largely invoked to provide the structural basis for the third-dimension static deformation of caveolae [30].

Physico-chemical studies on asymmetric model systems are very scarce, due to the difficulty of both realising an asymmetric experimental model of known composition and providing a suitable mean for spectroscopic observation. Older attempts to realise asymmetric phospholipid-ganglioside vesicles revealed an influence of gangliosides on the bilayer local structure, stability and permeability [92].

Nonetheless, the use of such asymmetric model systems is not at all widespread, due to the difficulty of realising artificial membranes with well defined heterogeneous composition. Accordingly, a suitable framework for data and theoretical treatment has not been developed for a long time. Promising experimental models bearing forced asymmetry of gangliosides are single supported or floating bilayers [93,94].

Recently molecular dynamics simulations have been tried [95,96] that still have to face the induced curvature and the long equilibration times required by ganglioside molecular rearrangement, eventually driving to glassy states, in the real or in the simulated membrane.

A less immediate effect of the tranverse distribution of gangliosides has been found to be played on the dynamics of mixed nanoscale membranes. In fact they gain in deformability by forced totally asymmetric disposition of GM1, while suffer from increasing bending rigidity by conventional mixing [15]. This is major feature, as it involves not only the structural organisation of the membrane, but also its local dynamic response.

7. Other remarks

All along the discussion, the word *order* has been used quite freely, as it reflects different aspects in different contexts. *Ordered* is "stretched chains in the lipid core", as in the liquid-ordered phase, and *ordered* is a colloidal liquid crystal originating in a Bragg diffraction pattern. On the membrane, *ordered* is also a regular topography decorating the surface, or a part of it. Besides being generated by "smooth" thermodynamic evolution, surface topography could result from dynamical arrest, that is, hindrance and shape of molecules can be so pronounced that they cannot diffuse as points on the surface, a matter of major concern in the case of bulky gangliosides. In other words, one may wonder whether a 2D glass transition may occur at least in confined regions of the membrane surface, domains or subdomains, where ganglioside density eventually satisfies the static rules for a 2D glass transition [97], a headgroup surface fraction around 60–70%. This possibility is highly suggestive for gangliosides, as sugars themselves display glass transitions [98], also affecting membrane phase stability [99]. The solid-disordered phase of GM3 [74], for example, could be originated by such an event, the classification of which as a 2D glass transition would nonetheless require direct access to the dynamics of the system [100].

Finally, a lot can be learnt on ganglioside behaviour and structural effects from monolayers and supported bilayers, with the usual warnings regarding possible artefacts induced by the presence of probes [101]. Nonetheless, we believe that their structural role can be fully exploited only if the third dimension is allowed, wrapping them-selves, and the membrane surface, onto non-euclidean geometries.

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