Current Opinion in Colloid & Interface Science 26 (2016) 58–65

Contents lists available at ScienceDirect

Current Opinion in Colloid & Interface Science

journal homepage: www.elsevier.com/locate/cocis

# Self-organization of nucleic acids in lipid constructs

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ARTICLE INFO

*Article history:*
Received 18 August 2016
Accepted 2 September 2016
Available online 28 September 2016

*Keywords:*
Self-assembly
Gene delivery
Lipid–DNA
Lipid–siRNA

ABSTRACT

Lipids and nucleic acids (NAs) can hierarchically self-organize into a variety of nanostructures of increasingly complex geometries such as the 1D lamellar, 2D hexagonal, and 3D bicontinuous cubic phases. The diversity and complexity of those lipid–NA assemblies are interesting from a fundamental perspective as well as being relevant to the performance in gene delivery and gene silencing applications. The finding that not only the chemical make of the lipid–NA constructs, but their actual supramolecular organization, affects their gene transfection and silencing efficiencies has inspired physicists, chemists, and engineers to this field of research. At the moment it remains an open question how exactly the different lipid–NA structures interact with cells and organelles in order to output an optimal response. This article reviews our current understanding of the structures of different lipid–NA complexes and the corresponding cellular interaction mechanisms. The recent advances in designing optimal lipid–based NA carriers will be introduced with an emphasis on the structure–function relations.



## 1. Introduction

Lipids are a class of organic compounds possessing both hydrophilic and hydrophobic moieties. The amphiphilic nature of lipids leads to molecular self-assembly behavior in solutions, forming various nanostructures of liquid crystalline nature. The characteristics of such assemblies that they are *nano-sized* and *structured* have led to numerous practical applications in pharmaceutical and biomedical technologies. Especially, the ability of lipid self-assembled aggregates to encapsulate nucleic acids (NAs) have earned researcher's attention to exploit them as gene delivery vehicles (vectors) as early as in 1979. The advantages of these vectors include the ease of preparation, low immunogenicity, and ability to transfer a large amount of NA. However, low gene delivery efficiency of those vectors has limited their therapeutic applications, provoking the improvements of the vectors by rational design. The rational design of gene delivery vectors requires a coherent understanding of their structures and interactions with cells. The step-by-step mechanisms of actions are still not clearly understood but we know from substantial research efforts that the structure of lipid–NA complexes is one of the important parameters determining the gene delivery efficiency. In addition, such systems have attracted the attention of a broad range of disciplines as the assembly of lipid–NA constructs is orchestrated by a balance of forces of different natures such as long range electrostatic attraction/repulsion and short range repulsions, many of which remain poorly understood at a very fundamental level [1–4]. The current research efforts are directed towards clarifying the interplay between distinct nanoscale structures of lipid–NA complexes and their interactions with cells. In line with this research trend, we provide an overview of the development of the lipid–NA complexes focusing on the structural aspect, and deliver insights for the future research in this field.

In the first part of this review, we present an introduction to the general principles governing lipid self-assembly behavior. This section will help the reader to make the link between the molecular structure of lipids and the resulting self-assembly morphology of lipid–NA complexes. A chronological account of the various structures of lipid–NA constructs is provided as well as how they correlate to cellular interactions and gene delivery/silencing efficacy. Finally, we propose a number of future research directions and applications for lipid–NA complex materials.

## 2. Lipid self-assembly

Lipid molecules are mostly known to self-organize into bilayer structures resembling that of plasma membranes. However, depending on a number of intrinsic (e.g. molecular packing and membrane curvature) or extrinsic (salinity, solvent properties, or external cues) properties, lipids offer a rather diverse design space of nanostructures. Examples include 2D hexagonal phases and 3D cubic phases of multiple symmetries [5].

The pioneering work on fundamental theory of self-assembly of amphiphiles was proposed by Tanford [6] who quantitatively modeled the standard free energy change associated with the transfer of singly dispersed free amphiphiles in solution to self-assembled aggregates. He introduced the concept of opposing energy contributions; a negative free energy contribution arising from the hydrophobic effect and a positive free energy contribution due to the repulsive interactions emanating

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http://dx.doi.org/10.1016/j.cocis.2016.09.006
1359-0294/

from lipid head groups. The hydrophobic interaction drives the transfer of the hydrophobic lipid tail that disrupts the H-bonding water network to the aggregate core which possess the hydrocarbon-like environment. While the hydrophobic effect favors the formation of aggregates, the assembly of lipid molecules entails the repulsive interactions such as steric interactions for all types of lipid head groups, dipole–dipole interactions for zwitterionic head groups, and ion–ion repulsions for ionic head groups. The competition between the hydrophobic effect and repulsive interactions, therefore, explains why the self-assembly process happens, how it is highly tunable by environmental conditions, and why the resulting aggregates have a finite size. Although Tanford's model explains the basic features of self-assembly, it could not predict the final shape of the equilibrium aggregates.

Israelachvili et al. [7] further developed the framework of the Tanford free energy model by combining the concept of a molecular packing parameter with thermodynamics to predict the shape of the self-assembly aggregates in equilibrium. To parameterize the geometric constraints imposed by molecular packing, a specific packing parameter $P$ was introduced. A molecular packing parameter is defined as $P = v/(al_c)$, where $v$ = the volume of the hydrocarbon, $a$ = the effective area of the head group, and $l_c$ = the effective length of the lipid tail.

The magnitude of packing parameter is related to the shape of aggregate formed. Cone-shaped lipids with bulky head group (e.g., lysophosphocholine or LPC) with small $P$ (0–1/3) tend to form spherical aggregates such as micelles. Truncated cone-shaped lipids with $P$ value between 1/3 and 1/2 organize into cylindrical micelles, and those with packing parameter between 1/2 and 1 prefers to form flexible bilayers or vesicles. Cylinder-shaped lipids (e.g., dioleylphosphocholine or DOPC) tend to form the flat bilayer with nearly zero curvature, i.e. lamellar phase ($P \approx 1$). For lipids with small effective head group area such as dioleylphosphatidylethanolamine or DOPE, the relative tail splay becomes wide ($P > 1$), leading to non-bilayer lipid phases such as an inverted hexagonal phase. Fig. 1 schematically represents typical phases obtained with lipid systems.

Although the general principles of lipid self-assembly could be well understood from the pioneering work by Tanford and Israelachvili, it could not capture the complete morphology of all lipid structures. As an alternative to the packing parameter, Gruner [8] explained nonlamellar phase formation in terms of spontaneous curvature. If a molecule has a preferred average geometry, the deviations from the preferred geometry yield a bending energy cost. Often, it is necessary to think about the stabilization of the different lipid phases in terms of lipid membrane elasticity that was appropriately described by Helfrich [9,10]. The Helfrich equation is expressed as the following: the Helfrich energy ($E$) per area ($A$) $= 0.5\kappa(C - C_0)^2 + \kappa_G C_1 C_2$ where $\kappa$ and $\kappa_G$ are the bending and the Gaussian modulus respectively. The spontaneous curvature $C_0$ represents a tendency of a flat monolayer to bend spontaneously. Practically, it means a stress-free state of a monolayer at given geometry. The first term thus describes the energetic cost of bending a lipid layer from its spontaneous curvature $C_0$ to the deformed curvature $C$. The second term accounts for the topology of the lipid layer where $C_1$ and $C_2$ are two principal curvatures of the lipid layer plane. The Helfrich equation becomes useful when describing for example lipid bicontinuous cubic phases that were firstly introduced by Luzzati [11]. The failure of explaining the formation of biocontinuous cubic phases by the packing parameter concept arises from the negligence of membrane curvature arguments with global packing constraints. While the packing parameter $P$ treats geometrical constraints locally, i.e. at the single-molecule level, the concept of a membrane curvature extends the packing constraints to be macroscopic. In general, the shape of the resulting self-assembly aggregate can be predicted as a function of the spontaneous curvature $C_0$ of membranes.

## 3. Interactions between nucleic acids and lipid aggregates

To successfully deliver nucleic acid-lipid complexes to the target site of delivery, stable loading of nucleic acids into lipid formulations should be achieved first. DNA-lipid binding and DNA condensation are the first stage of the gene delivery process. In this context, we will take a look into electrostatic interactions that mainly govern the DNA-lipid binding process and DNA condensation. The hydration of the nucleic acid-lipid complexes also comes into play in determining the self-assembled structure of nucleic acid-lipid complexes. The finite amount of water included in the complexes exerts repulsive forces preventing the collapse of the complexes. As the interactions among nucleic acids, lipid headgroup, lipid tail, and surrounding water molecules occur in a highly cooperative manner, it is hard to separate out the individual component of such interactions. However, the examination of two main interactions between nucleic acids and lipid aggregates will help us to gain a better understanding of the structure of lipid–NA complexes.

### 3.1. Electrostatic interaction

DNA is a negatively charged polyelectrolyte whose conformation is influenced by the ionization of phosphate groups in its backbone. When cationic lipid such as 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) or 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) binds to the extended DNA backbone, the positive charge of the lipid headgroup neutralizes the phosphate groups on the DNA leading to DNA condensation. During such lipid–DNA binding process via electrostatic interactions, counterions that were both bound to DNA and charged lipids become released in bulk solution, resulting in a large increase in entropy. According to a mathematical model of lipid–DNA binding thermodynamics [12], lipid–DNA interaction is mainly mediated by two contributions: 1) a translational entropic gain due to counterion release and 2) an increase in entropy due to additional disorder of the lipid aliphatic chains. The direct evidence of counterion release upon CL-DNA condensation was demonstrated by Wagner et al. [13] with the conductivity measurement of the solution before and after the CL-DNA complex formation.

The electrostatic interaction is strongest in the absence of salt ions, i.e. when there is no electrostatic screening effects. Thus use of salt ions with different charge and concentration can modulate the strength of the electrostatic interaction bringing additional changes to the complex structure. For example, addition of salt ions to DNA-surfactant complexes has been reported to increase the uptake of water where the driving force of swelling is based on the higher contribution of salt to the osmotic pressure accompanied with decreased electrostatic attraction [14].

The electrostatic interactions between DNA and *cationic/zwitterionic* lipid membranes are significant when it comes to the molecular mobility and the packing of lipids. In DNA–lipid complexes reported by

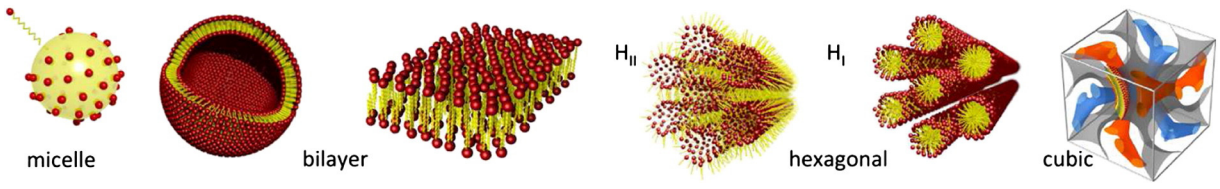


**Fig. 1.** Schematic representation of various lipid self-assembled structures. Lipids in the presence of water can form micelle, liposome, inverse and normal hexagonal phases, bicontinuous cubic phase, respectively from left to right.