REVIEW ARTICLE

Lipid-based liquid crystalline materials in electrochemical sensing and nanocarrier technology

Martina Zatloukalova[1] · Lukasz Poltorak[2] · Renata Bilewicz[3] · Jan Vacek[1]

Abstract

Some biologically active substances are unstable and poorly soluble in aqueous media, at the same time exhibiting low bioavailability. The incorporation of these biologically active compounds into the structure of a lipid-based lyotropic liquid crystalline phase or nanoparticles can increase or improve their stability and transport properties, subsequent bioavailability, and applicability in general. The aim of this short overview is (1) to clarify the principle of self-assembly of lipidic amphiphilic molecules in an aqueous environment and (2) to present lipidic bicontinuous cubic and hexagonal phases and their current biosensing (with a focus on electrochemical protocols) and biomedical applications.

Introduction

Surfactants and amphiphilic substances (lipids) are not only basic biomolecules of the human body, but they are also one of the main components of pharmaceutical, cosmetic, and food industry products. As a class of biomolecules, they are a source of energy, and play a number of vital functions, such as cell differentiation, signal transduction, organ protection and barrier functions, and the synthesis of essential biomolecules including hormones and bile acids [1]. Lipids are amphiphilic substances that contain hydrophilic and hydrophobic moieties in their molecular structure. The non-polar bonds of the hydrophobic part of the molecule are the reason for their limited solubility in aqueous media. The solubility of lipids and other amphiphilic molecules is characterized by a critical micellar concentration (CMC) [2]. When the CMC of individual monomers is exceeded, higher lipidic structures such as micelles, liposomes, lipidic nanoparticles, and lipidic lyotropic liquid crystals are formed in an aqueous medium [3].

At the turn of the nineteenth and twentieth centuries, the Czech-Austrian biologist Reinitzer [4] discovered that the cholesteryl ester of benzoic acid passes into a liquid state at a temperature of 145 °C; up to 179 °C, it has a milky coloration; and from 179 °C upwards, it is a clear liquid. His pilot studies were followed by the physicist Lehmann [4], who called these states of mater/substances "mesophases" (later, liquid crystals). Liquid crystals are a transition between liquid and solid crystalline states, and thus, they have the properties of both solid substances (ordered and oriented molecules) and liquids (mobility, fluidity). Liquid crystals can be obtained by dissolving a solid substance in a solvent (lyotropic liquid crystals) or by melting (thermotropic liquid crystals). Liquid crystals can also form in an aqueous medium from some lipids [4].

A lipid-based lyotropic liquid crystalline phase is a material that imitates biological membranes. Thus, it represents a suitable matrix for stabilizing hydrophilic, hydrophobic and amphiphilic biologically active compounds, which are often relatively unstable with limited or no solubility in aqueous media. The incorporation of these relatively unstable molecules into carrier media expands the range of possibilities for their stabilization,

✉ Martina Zatloukalova
  martina.zatloukalova@upol.cz

✉ Jan Vacek
  jan.vacek@upol.cz

[1] Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Hnevotinska 3, 775 15 Olomouc, Czech Republic

[2] Electrochemistry@Soft Interfaces Team, Department of Inorganic and Analytical Chemistry, Faculty of Chemistry, University of Lodz, Tamka 12, 91-403 Lodz, Poland

[3] Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland

transport, and controlled (frequently targeted) release. The techniques for nanoencapsulation are constantly evolving, providing new options for the preparation and application of targeted formulations [5].

Over the last few decades, there has been a focus on developing new ways of targeted drug administration. Ideally, the transport and delivery system of such drugs should meet several prerequisites. It should have a high capacity for incorporating substances, be stable and biocompatible, allow for the controlled release of substances, and be targeted at the site of action [6]. Current conventional forms of medication, including controlled dosage release, do not meet all of these preconditions. On the other hand, a number of nanoforms (including polymer nanoparticles and nanocapsules, liposomes, solid lipidic nanoparticles, phytosomes, nanoemulsions, and others) have significant advantages, including increased solubility and bioavailability, stability, and improved tissue distribution. Nanoforms with incorporated biologically active substances thus have the potential to increase the bioavailability and stability parameters of drugs and biologically and pharmacologically active substances in general. Moreover, the combination of drugs can be delivered in one cubosome or hexosome, lipidic cubic phase (LCP)-based nanoforms, and by adding special groups to the nanoform, addressable delivery vehicles can be achieved.

In addition to drug delivery systems, the use of lipid membranes and functional layers in biosensors is frequently discussed today [7, 8]. Biosensors based on lipid membranes make it possible to investigate the properties of membranes and membrane proteins, but also investigate the influence of biologically active substances on their function and stability. The stability of lipid membrane-based biosensors limits their practical use [9]. There are several approaches to achieving stable lipid membranes on electrode surfaces: solid supported lipid bilayers, polymer cushioned bilayers, hybrid lipid bilayers or multilayers, the classical black lipid membrane, and others [10]. The above-mentioned instability could also be overcome by using LCP-based layers or nanoforms. In addition, the multifunctionality of cubosomes/hexosomes could open up new possibilities, as was recently reported with loading enantiomeric ligands, which resulted in the preparation of chiral LCP systems [11, 12].

The aim of this review is to briefly describe the preparation, classification and application of lipid mesophases, the incorporation of proteins into LCP, and the formation and applications of lipid nanoparticles (cubosomes and hexosomes). The future directions of this research are also highlighted, including a strategy towards lipid-based object detection at polarized liquid–liquid interfaces.

Lipidic mesophase

The spontaneous arrangement of amphiphilic molecules into organized structures is one of the features of many biological systems such as the cell plasma membrane, endoplasmic reticulum, Golgi apparatus, and the densely convoluted mitochondrial membrane. This is the inspiration for the development of new biomimetic materials [5]. The goal of self-assembled formations is to achieve an energy-advantageous state. One of the main drivers of the formation of these supramolecular structures is the hydrophobic effect. However, there are many factors that determine and influence the structure and stability of individual self-assembled formations. In this regard, a decisive role is played by the concentration and shape of the amphiphile. If the concentration of the amphiphile is equal to or higher than the CMC, and the temperature is higher than the critical micellar temperature (also known as the Krafft temperature), the formation of micelles occurs. Amphiphiles differ mainly in the size and shape of the hydrophilic and hydrophobic molecular moieties, which is reflected in their spatial arrangement (Fig. 1).

Surfactants tend to form self-assembled cone-shaped type 1 structures, while molecules that have a smaller polar part, such as lipids, tend to form inverse micellar phases of type 2. Biological membranes composed of a wide range of molecules of varying shapes form dynamic self-assembled formations, including local planar/lamellar structures [15]. The shape of self-assembled formations can be qualitatively described according to Israelachvili et al. [2]. This theory is based on a dimensionless parameter, the so called critical packing parameter (CPP). The CPP is defined according to the following equation:

$$CPP = \frac{v}{a_0 l_c}$$

where v is the volume of the hydrophobic tail, a_0 is the area of the head group, and l_c is the length of the hydrophobic tail. Spherical and cylindrical micelles are formed both in the self-arranged phase of type 1 and in the inverse micellar arrangement of type 2.

Amphiphiles in an aqueous medium have a spatial arrangement, with the basic lipidic phases designated as lamellar, hexagonal, and bicontinuous cubic phases. With specific components added to the system, other phases can also form [15]. Most common mesophase lipidic structures are based on 1-monoacylglycerol (1-monoolein) and phytantriol, and these are shown in Fig. 2.

1-Monoolein (1-(cis-9-octadecenoyl)-*rac*-glycerol, MO) is a clear viscous substance with a characteristic odor [16]. MO is insoluble in water, but dissolves well in oil and lower hydrocarbons such as chloroform. In particular, due to its
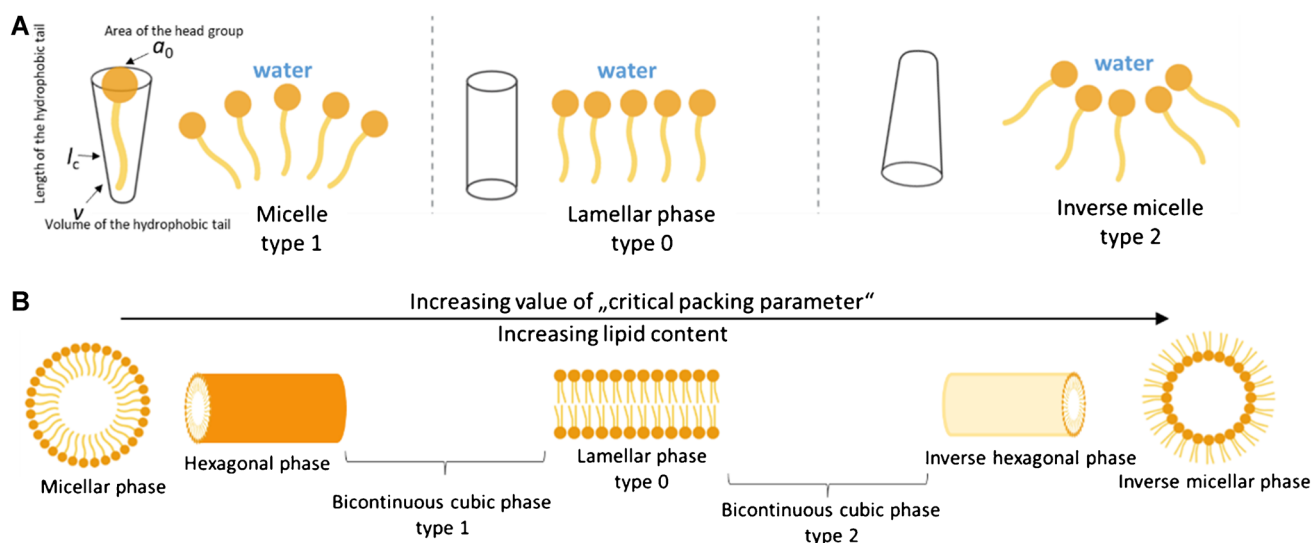
Fig. 1 **A** Shape and **B** type of self-assembly systems of amphiphilic molecules (schematically) after contact with water. Modified as described in refs [13, 14]
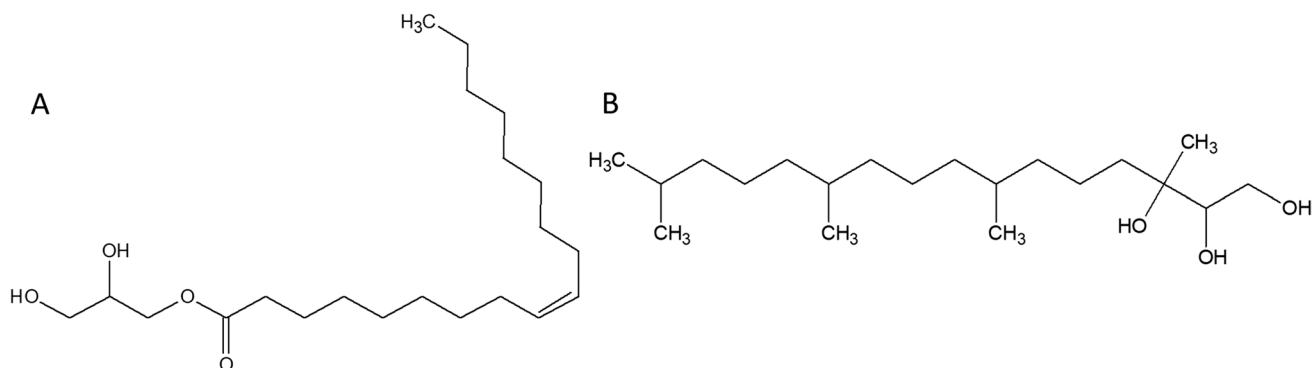

Fig. 2 Structure of **A** 1-monoolein and **B** phytantriol

high solubility in oil, MO is used as a food emulsifying agent. It is non-toxic, biodegradable, and biocompatible [13]. The properties of amphiphilic molecules are crucial for the final composition and structure of the individual phases, of which a bicontinuous cubic phase in particular has a high degree of spatial organization [17, 18]. An important condition for the reproducibility of the formation of these spatially ordered phases is the precise definition of the default components used and their ratio. A lipidic cubic phase (LCP) is one of the many liquid crystalline phases that spontaneously form when lipids are mixed with water under appropriately selected conditions: water-to-lipid ratio and temperature range. A schematic presentation of the procedure used for the preparation of LCP is shown in Fig. 3A. LCP is a thermodynamically stable, self-assembled lipidic

phase with unique properties and structure. The cubic phase consists of two continuous phases, one of which is a lipid bilayer, and the other is formed of aqueous channels. The LCP is characterized by a crystallographic spatial arrangement with *Im3m* (primitive), *Pn3m* (double diamond), or *Ia3d* (gyroid) symmetry [13]. In the *Im3m* phase, the water channels meet in six-way junctions at an angle of 90°. The *Pn3m* phase is characterized by a four-way crossing of water channels at an angle of 109.5°. Cryo-TEM images of LCP nanoparticles based on MO and phytantriol, both with *Pn3m* symmetry, are shown in Fig. 3B, C. Three-way junctions intersecting at an angle of 120° are typical for the *Ia3d* phase [13]. The cubic phase is an attractive tool for a number of different biosensing, biomedicine, and food industry applications (Fig. 4).

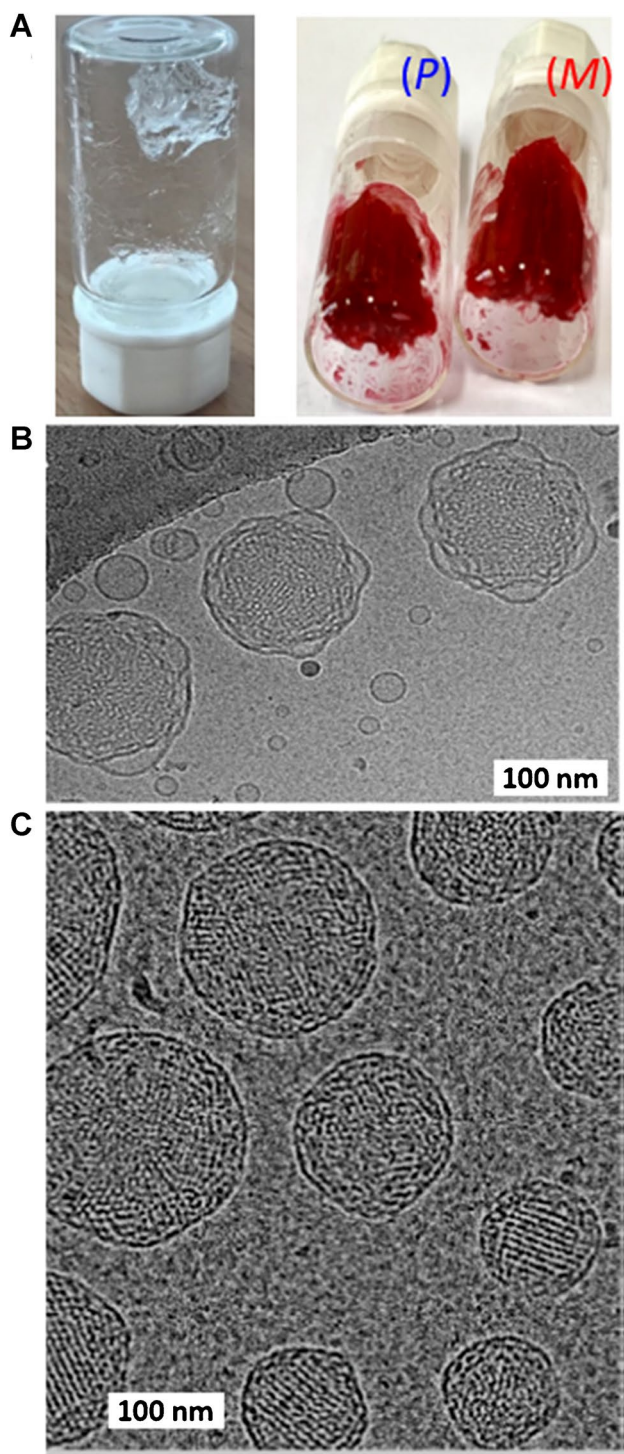**Fig. 3 A** Pure lipidic cubic phase (left) and lipidic cubic phase loaded with *P* and *M* enantiomers of flavo[7]helicene (right); for more details, see ref. [12]. Cryo-TEM images of **B** 1-monoolein- and **C** phytantriol-based cubosomes as described in more detail in refs [19, 20]

Integration of proteins into the lipidic cubic phase

The cubic phase has been used for more than two decades as a matrix for the crystallization of integral membrane proteins. Landau and Rosenbusch were the first to use a LCP for the crystallization of a membrane protein in 1996 [22]. Today, there are more than 700 proteins in the protein databank (PDB) that have been crystallized in a LCP (crystallization *in meso*) [25–31]. These include a number of enzymes, transporters [32], channels, receptors [33], and structural proteins [34]. Protein crystallization in a LCP made it possible to elucidate the structures of several microbial rhodopsins and receptors associated with G-proteins. Biomimetic membranes such as LCPs provide proteins with a more natural membrane environment, as opposed to the artificially created environment associated with the presence of detergents [27, 35, 36]. An example of the use of a LCP for the crystallization of polar, water-soluble proteins is lysozyme. Unlike membrane bacteriorhodopsin [37, 38], lysozyme crystallizes independently of the lipidic phase type [23]. LCP technologies for structural studies of membrane proteins such as bacteriorhodopsin or gramicidin were reviewed by Cherezov [39]. The most common cubicon method for the incorporation of membrane proteins was described by Ma et al. [40]. The organization of proton pumps, lipids, and water in cubic mesophase crystals was discussed by Belrhali et al. [41].

The functional properties of the proteins are retained in the lipidic environment, as shown in several reports [42]. They can therefore be used as active components of lipid-liquid-crystalline films, e.g., deposited on solid surfaces. A viscous, stable three-dimensional lipid bilayer with incorporated membrane proteins/redox probes is easily applied to an electrode surface [43]. Less recent applications in sensing are presented in refs [10, 44]. Fructose dehydrogenase (FDH) was reconstituted in a monoolein cubic mesophase and used for the electrochemical determination of fructose [45]. Mezzenga et al. confirmed in a spectrophotometric study that *in meso*-immobilized FDH has improved stability compared to other matrices or solutions [46].

In a recent study, we reconstituted and showed the activity of the Na^+/K^+-ATPase (sodium–potassium pump, NKA) in a MO-based LCP for the first time [47]. The reconstitution and chloride transport of the chloride transporter protein EcClC in the LCP were described by Speziale et al. [48] and by ourselves [49], and the efficiency of chloride transport was studied using inhibitors and activators of the protein. Active gating was demonstrated for the glucose transporter protein [50]. Lipidic liquid-crystalline mesophases are also used for protein biochip development [51].

Fig. 4 Scheme depicting properties and applications of LCP (lipidic cubic phase). Based on recent reviews in the field: drug delivery [21], crystallization [22, 23], biosensors [10], and a very recent review [24]
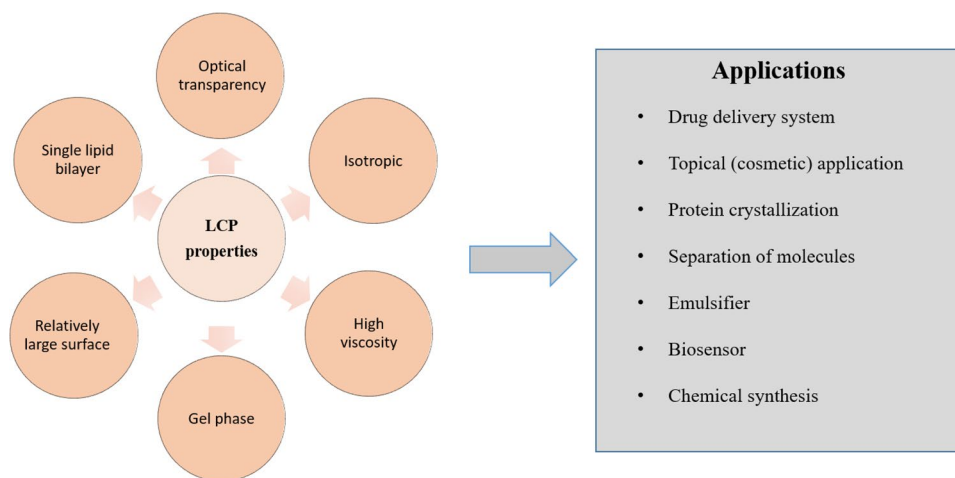
Table 1 Electrochemical enzyme biosensors based on LCP

Enzyme/protein	Method	Electrode	Ref.
Glucose oxidase, ceruloplasmin	Amperometry	Platinum disk	[53]
Hemoglobin	CV, amperometry	Glassy carbon electrode	[54]
Glucose oxidase, lactate oxidase, urease, creatinine deiminase	Amperometry, potentiometry	Platinum electrode, pH electrode	[52]
Glucose oxidase, pyranose oxidase, laccase	CV	Glassy carbon electrode	[55]
Glucose oxidase	CV	Carbon electrode	[56]
Na^+/K^+-ATPase	SWV	Glassy carbon electrode	[47]
Ethanol dehydrogenase	CV, DPV	Glassy carbon electrode	[57]
Laccase [#]	Chronoamperometry, CV, AC impedance	Modified glassy carbon electrode	[58]
Cellobiose dehydrogenase	CV, DPV	Modified glassy carbon electrode	[59]
Cholesterol oxidase	CV	Glassy carbon electrode, gold electrode	[60]
Bilirubin oxidase	CV	Carbon rotating disk electrode	[61]

CV, cyclic voltammetry; *SWV*, square wave voltammetry; *DPV*, differential pulse voltammetry; [#]discussed with Rowinski et al

Polar analytes reside in the aqueous channels of lipidic mesophases, providing free access to the electrode surface and proteins. These substances easily communicate with the electrode directly or *via* an electroactive probe [10]. In 1994, Razumas et al. [52] were the first to report on the preparation of LCP biosensors. The first biosensors based on enzymes incorporated into a LCP were designed to determine glucose, lactate, urea, and creatinine. Current responses dependent on H_2O_2 oxidation were detected amperometrically. Table 1 summarizes published electrochemical enzyme biosensors based on a LCP.

These studies show that proteins can be incorporated into the lipidic phase at relatively high concentrations, and they readily interact with the surface of the electrode. However, what should be taken into account is the influence of physico-chemical factors such as the ratio of water to lipid in the mesophase, temperature, pressure, lipid composition, and the presence of other substances. These factors could influence the phase transition of the LCP, lead to its destabilization, and promote the subsequent release of incorporated enzymes due to the transition of the LCP to a hexagonal or lamellar phase.

In addition to protein crystallization and biosensor construction, a LCP can also be used as a matrix for research into the stability, activity, and interaction of proteins with ligands [47, 62, 63]. One example is a stability study of the above-mentioned NKA. NKA carries sodium and potassium ions against their concentration gradient using energy from ATP hydrolysis. In this experiment, after 14 days, the NKA activity in the LCP was still 60% of its maximum activity, while it was no longer active in the parallel experiment incubated in an aqueous environment [47]. A model example of research on the redox behavior of proteins incorporated into a LCP are studies conducted using cytochrome *c* [63–65]. The interaction of cytochrome *c* with a LCP mimicking the environment of the inner mitochondrial membrane was investigated using FTIR spectroscopy, differential scanning calorimetry, and electrochemical techniques. The diffusion

coefficient of cytochrome *c* was determined using electrochemical methods, and it showed very limited protein mobility in the lipid environment; more details can be found in a relatively recent review [36].

Electroactive probes and lipidic cubic phase

Barauskas et al. [66] prepared an electrochemically active cubic phase containing various types of amphiphilic substances (ferrocenes). A fold decrease in the diffusion coefficient of amphiphiles incorporated into the LCP was observed compared to the diffusion coefficient determined in acetonitrile solutions. Rowinski et al. [43] investigated the behavior of hydrophilic probes in a cubic phase using cyclic voltammetry, differential pulse voltammetry, and chronocoulometry. The diffusion coefficients for $[Ru(NH_3)_6]^{3+}$ and benzoquinone in the LCP were found to be lower and in accordance with the diffusion coefficient determined in solution. Similarly, Kostela et al. [67] determined the diffusion coefficient of hydrophilic, hydrophobic, and amphiphilic electroactive probes in a LCP. The diffusion coefficients for these electrochemically active species were also determined in a hexagonal phase using electrochemical and impedance methods [68]. In-depth experimental and theoretical studies of diffusion in a LCP have been reported more recently [69, 70].

Lipidic nanoparticles, general aspects

New lipid-based nanocarriers are constantly being sought and developed for the design of biosensors and the development of transport and application systems. One of the key advantages of using lipidic nanoparticles is the effective solubilization of poorly water-soluble substances, as demonstrated, for example, with curcumin [71] and quercetin [72]. The most explored lyotropic nanostructure carriers include cubosomes and hexosomes, which are defined as colloidal nanoparticles with internal bicontinuous cubic and hexagonal structures. This group of colloidal dispersions is called ISAsomes (internally self-assembled "somes" or particles).

In addition to cubosomes and hexosomes, ISAsomes include micellar cubosomes [73]. The first mention of the existence of cubosomes dates back to the 1980s, when Larsson discovered that the dispersion of LCPs produces submicron particles with an identical internal arrangement to the parent cubic structure [74]. Cubosomes are highly stable nanoparticles formed from a lipidic cubic phase and stabilized by the outer layer. Compared to liposomes, cubosomes and hexosomes provide a significantly larger surface area (up to 400 m^2/g) for the incorporation of membrane proteins and small hydrophilic or hydrophobic molecules [75]. In general, there are two main approaches to the preparation of cubosomes, the "top-down" and "bottom-up" approach, both of which require the use of a suitable stabilizer (Fig. 5).

The "top-down" method, the most widely used and oldest technique used for the preparation of cubosomes [16], involves two main steps. First, the LCP is prepared, which is then homogenized/sonicated using high-energy pulses. Cubosomes prepared by the "top-down" method are stable against aggregation for up to 1 year. The disadvantage of this method is however the use of high-energy pulses, which can affect the activity of incorporated biologically active substances sensitive to elevated temperature [75].

The second method, commonly called the "bottom-up" method, involves the dispersion of a mixture containing lipid, stabilizer, hydrotrope, and an excess of water (Fig. 5) [76]. The hydrotrope is a key factor in this process and helps to solubilize lipids [77]. The most frequently used hydrotropes are urea, sodium alginate, sodium benzoate, or ethanol. The advantage of this approach is the use of less energy, so that it can also be applied for the preparation of cubosomes with thermounstable substances such as peptides and proteins [16]. The same preparation procedure can be used for hexosomes [78]. The LCP phases dispersed into nanoparticles used as drug nanocarriers were recently reviewed by Angelova et al. [79], Murgia et al. [80], and Tenchov et al. [81].

To prevent the aggregation of ISAsomes, stabilizing components have to be added during the synthetic procedure. Over the last decade, various types of stabilizers have been proposed for the preparation of cubosomes and hexosomes. The most common and effective of these surfactants is

Fig. 5 Schematic representation of "top-down" and "bottom-up" general approaches for preparation of cubosomes and hexosomes
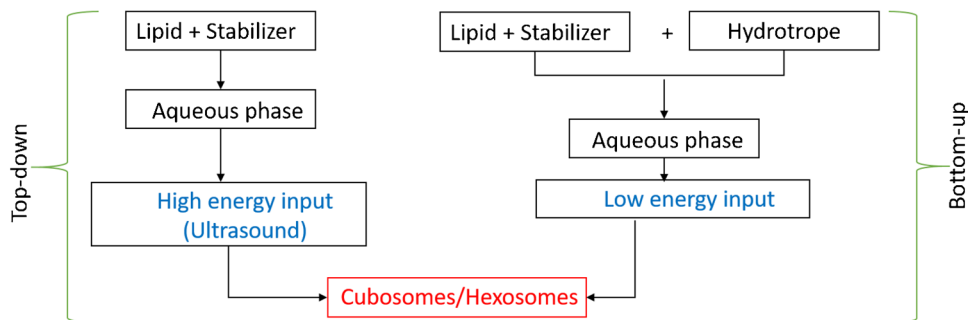
Poloxamer 407, which is commercially available under the name Pluronic F127 [82, 83]. In addition to Pluronic F127, other stabilizing agents have been proposed, including various co-polymers such as F128, F108 [84, 85], PEGylated lipids, or β-casein [86]. The choice of stabilizer for the preparation of ISAsomes is critical, as stabilizers can also affect the internal lipidic nanostructure and thus the affinity towards the cargo [87].

Liquid crystalline phases and their corresponding aqueous dispersions are characterized primarily by two techniques, namely, SAXS (small-angle X-ray scattering) and SANS (small-angle neutron scattering) [88]. Studies are mainly focused on describing the influence of physico-chemical factors on the structural properties of nanoparticles, including lipid composition, temperature, pH, pressure, and the effect of target substance incorporation. In addition to SAXS and SANS, cryo-TEM [89] and AFM [90] are also used for morphological characterization. Dynamic light scattering (DLS) is frequently employed for monitoring particle size. To demonstrate the use of nanocarriers for biosensor construction, we present work based on phytantriol-based cubosomes stabilized with F127. The solid gold surface of the sensor was modified through biotinylated lipids that were part of the cubosomes. The second set of cubosomes enriched with glycolipid (GM1) was then applied to the modified surface, and this led to the specific binding of cholera toxin B from solution [91].

Applications of cubosomes and hexosomes

These days, we are seeing a growing interest in the use of ISAsomes, especially cubosomes and hexosomes, as nanocarriers for sensing strategies and for drug incorporation, imaging probes, and antimicrobial peptides. Particular attention is paid to the solubilization and stabilization of biologically active substances, the influence of lipid composition, and the type and concentration of the stabilizer on the structural and morphological properties of these nanoforms. On the other hand, there have been few studies on the release of substances from cubosomes/hexosomes and factors affecting the stability of these structures, especially in a living organism [92, 93]. There is increasing interest in the influence of incorporated substances or cubosomes/hexosomes themselves on cell signaling pathways. The release of doxorubicin from the cubic lipidic phase depending on the change in pH (with respect to differences in pH of tumors *vs.* non-malignant tissue) was studied using electrochemical methods [94]. The bioavailability (cell uptake kinetics) and cytotoxicity of cubosomes have been investigated mainly *in vitro* on cell lines [83, 95, 96]. Cubosomes and hexosomes are beginning to be applied in theranostics. DLS, SAXS, and cryo-TEM methods have shown that hexosomes are able to incorporate both a fluorescent probe and the anticancer

drug camptothecin into their structure. Fluorescence microscopy has shown that the HeLa cell line is able to accumulate modified hexosomes. For fluorescence microscopy, non-toxic concentrations of modified hexosomes were used [97]. Cytryniak et al. [19, 98] were the first to demonstrate the use of cubosomes in radiotherapy in combination with chemotherapy. MO-based cubosomes were modified by incorporating the anticancer drug doxorubicin and a commonly used radionuclide. The cytotoxicity of the modified cubosomes was tested on HeLa cells. Cubosomes modified with doxorubicin and the radionucleotide were shown to be more toxic than cubosomes alone or cubosomes with an incorporated chemotherapeutic/radionucleotide. The combination of cryo-TEM and SAXS methods was used to examine the impact of blood plasma on the size, structural, and morphological properties of cubosomes over time [99]. However, few other studies have focused on the stability of lipidic cubic and hexagonal nanoparticles in the bloodstream, structural transformations of cubosomes/hexosomes after contact with cell membranes, blood cells, or proteins, or their cell uptake. The structural arrangement of bicontinuous cubic and hexagonal phases allows for a gradual release of incorporated substances. Bicontinuous cubic nanostructures have also been shown to have mucoadhesive properties [100, 101]. The use of these 3D-nanolipidic structures was mainly intended for the oral, subcutaneous, transdermal, and periodontal administration of biologically active substances. Lipidic liquid crystalline phases are highly viscous and therefore have limited use as intravenous nanocarriers. Cubosome/hexosome suspensions are much less viscous and therefore much more convenient for drug delivery. The most important aspect of these drug delivery studies is related to the degradation of lipidic nanoparticles in the biological environment as a result of interactions with, e.g., enzymes and macrophages or other species present in the biological medium [102]. An interesting future direction of research could also be the use of lipidic vehicles for the incorporation of biologically active fatty acids and lipids [103]. A more detailed overview of the use of cubosomal/hexosomal lipidic structures based on MO or phytantriol for targeted drug delivery systems is provided in the following publications [5, 16, 73, 75, 78, 104].

Future directions: lipid-based objects and polarized liquid–liquid interfaces

Electrochemical studies of lipid-based objects, including lipidic nanoparticles and micelles, should go beyond configurations involving the utilization of solid electrodes [105]. We postulate that the concept introduced by Laborda et al. [106] and further studied by a few other groups [107–109], which describes the single fusion events of an emulsion

Fig. 6 i Schematic representation of concept assuming that liposome approaching liquid–liquid interface, upon interfacial adsorption, will fuse and release its cargo. **ii–v** are possible lipid-based object cargo—salt dissociated into ions/charged chemical species—interfacial ion transfer reactions
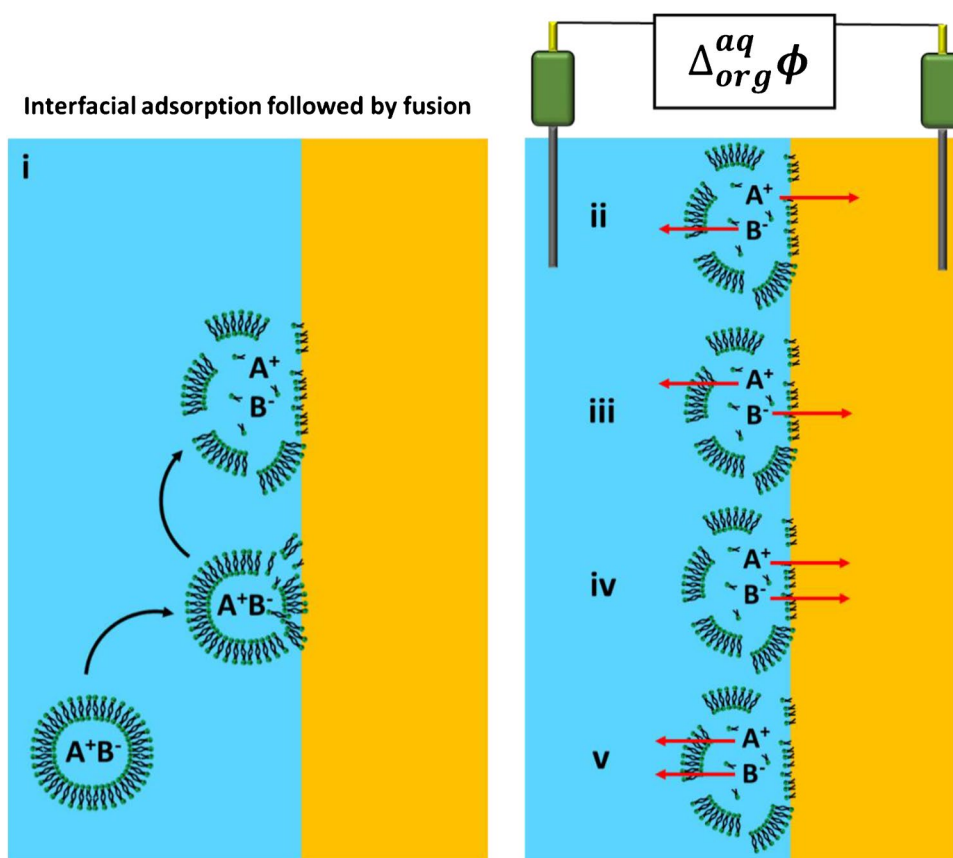
droplet hitting a polarized liquid–liquid interface, can be further developed and adapted to study the interactions of lipid-based objects with soft interfaces. This aspect is visualized in Fig. 6. One can imagine that the lipidic object, e.g., a liposome, when placed next to the electrified liquid–liquid interface (either bare or modified with a lipid monolayer [110]), can undergo fusion and the release of its cargo. When the Galvani potential difference ($\Delta_{org}^{aq}\phi$) is fixed to a value higher than $\Delta_{org}^{aq}\phi_{A^+}$ and $\Delta_{org}^{aq}\phi_{B^-}$ (see Fig. 6, ii), it is expected that cationic species will transfer to the organic phase, whereas anions will remain in the aqueous phase. When $\Delta_{org}^{aq}\phi$ is lower than $\Delta_{org}^{aq}\phi_{A^+}$ and $\Delta_{org}^{aq}\phi_{B^-}$ (see Fig. 6, iii), it is only the anion that should transfer to the organic phase; cations will stay in the aqueous phase. Both species are expected to undergo interfacial ion transfer when $\Delta_{org}^{aq}\phi$ is higher than $\Delta_{org}^{aq}\phi_{A^+}$ and lower than $\Delta_{org}^{aq}\phi_{B^-}$ (see Fig. 6, iv). The opposite situation, i.e., when $\Delta_{org}^{aq}\phi$ is lower than $\Delta_{org}^{aq}\phi_{A^+}$ and higher than $\Delta_{org}^{aq}\phi_{B^-}$, restricts partitioning. In this way, a full range of electrochemical techniques can be applied to either study or control the ionic partitioning of the molecular cargo carried by lipid-based objects. Moreover, we believe that properly designed experiments, in which lipidic objects carry an ionic cargo, can be used to follow the impacts of the lipid-based objects with a liquid–liquid interface-, fusion efficiency-, and potential-dependent adsorption

of the lipid objects to a polarized junction. A polarized junction is a biointerface that mimics a real cell membrane with established cell potential. The impacts are detected in the form of changes in the electric properties of the interfacial region that follow the ionic currents originating from the interfacial charge transfer reaction involving the encapsulated molecular cargo.

Conclusions

Highly organized 3D-lipidic architectures can be used as matrices for the crystallization of integral membrane proteins or in the development of new lipidic nanoforms for both analytical applications and the targeted transport and stabilization of biologically active molecules. Knowledge of the optimal internal lipid arrangement and selection of suitable agents for the stabilization of nanocarriers is essential for designing a specific usable lipidic matrix. Although we understand the unique properties of cubosomes and hexosomes, there are still a number of questions about the fate of nanocarriers after their *in vivo* administration. Similarly, there is limited knowledge in the field of cell testing, including a deeper understanding of the mechanism of interaction with cell membranes and

receptors, the actual entry into cells, and the release of biologically active substances from nanocarriers. As for sensing and the development of LCP-modified electrodes or microarray detection surfaces, fundamental research of interfacial behavior would be an important direction in the future.

Abbreviations *AFM*: Atomic force microscopy; *CMC*: Critical micellar concentration; *CPP*: Critical packing parameter; *Cryo-TEM*: Cryo-transmission electron microscopy; *DLS*: Dynamic light scattering; *FDH*: Fructose dehydrogenase; *ISAsomes*: Internally self-assembled "somes" or particles; *LCP*: Lipidic cubic phase; *MO*: 1-Monoolein; *NKA*: Na$^+$/K$^+$-ATPase; *PDB*: Protein databank; *SANS*: Small-angle neutron scattering; *SAXS*: Small-angle X-ray scattering

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Kulkarni CV (2012) Lipid crystallization: from self-assembly to hierarchical and biological ordering. Nanoscale 4(19):5779–5791. https://doi.org/10.1039/C2NR31465G
2. Israelachvili JN, Mitchell DJ, Ninham BW (1976) Theory of self-assembly of hydrocarbon amphiphiles into micelles and bilayers. J Chem Soc Faraday Trans 72:1525–1568. https://doi.org/10.1039/f29767201525
3. Patra JK, Das G, Fraceto LF, Campos EVR, del Pilar R-T, Acosta-Torres LS et al (2018) Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnology 16(1):1–33. https://doi.org/10.1186/s12951-018-0392-8
4. Mitov M (2014) Liquid-crystal science from 1888 to 1922: building a revolution. Chem Phys Chem 15(7):1245–1250. https://doi.org/10.1002/cphc.201301064
5. Shanmugam T, Banerjee R (2011) Nanostructured self assembled lipid materials for drug delivery and tissue engineering. Ther Deliv 2(11):1485–1516. https://doi.org/10.4155/tde.11.105
6. Nazaruk E, Górecka E, Osornio YM, Landau EM, Bilewicz R (2018) Charged additives modify drug release rates from lipidic cubic phase carriers by modulating electrostatic interactions. J Electroanal Chem 819:269–274. https://doi.org/10.1016/j.jelechem.2017.10.057
7. Vacek J, Hrbac J (2020) Sensors and microarrays in protein biomarker monitoring: an electrochemical perspective spots. Bioanalysis 12(18):1337–1345. https://doi.org/10.4155/bio-2020-0166
8. Van Gool A, Corrales F, Čolović M, Krstić D, Oliver-Martos B, Martínez-Cáceres E et al (2020) Analytical techniques for multiplex analysis of protein biomarkers. Expert Rev Proteomics 17(4):257–273. https://doi.org/10.1080/14789450.2020.1763174
9. Nikoleli G-P, Nikolelis DP, Siontorou CG, Nikolelis M-T, Karapetis S (2018) The application of lipid membranes in biosensing. Membranes 8(4):108. https://doi.org/10.3390/membranes8040108
10. Nazaruk E, Bilewicz R, Lindblom G, Lindholm-Sethson B (2008) Cubic phases in biosensing systems. Anal Bioanal Chem 391(5):1569. https://doi.org/10.1007/s00216-008-2149-y
11. Vacek J, Zadny J, Storch J, Hrbac J (2020) Chiral electrochemistry: anodic deposition of enantiopure helical molecules. ChemPlusChem 85(9):1954–1958. https://doi.org/10.1002/cplu.202000389
12. Jakubec M, Novák D, Zatloukalová M, Císařová I, Cibulka R, Favereau L et al (2021) Flavin-helicene amphiphilic hybrids: synthesis, characterization, and preparation of surface-supported films. ChemPlusChem 86(7):982–990. https://doi.org/10.1002/cplu.202100092
13. Kulkarni CV, Wachter W, Iglesias-Salto G, Engelskirchen S, Ahualli S (2011) Monoolein: a magic lipid? Phys Chem Chem Phys 13(8):3004–3021. https://doi.org/10.1039/C0CP01539C
14. Zatloukalová M (2022) 3D lipidic matrix for incorporation and stabilization of biologically active molecules. Chem Listy (in Czech) 116(3):172–179. https://doi.org/10.54779/chl20220172
15. Kulkarni CV (2016) Lipid self-assemblies and nanostructured emulsions for cosmetic formulations. Cosmetics 3(4):37. https://doi.org/10.3390/cosmetics3040037
16. Gaballa SA, El Garhy OH, Abdelkader H (2020) Cubosomes: composition, preparation, and drug delivery applications. J Adv Biomed Pharm Sci 3(1):1–9. https://doi.org/10.21608/jabps.2019.16887.1057
17. Qiu H, Caffrey M (2000) The phase diagram of the monoolein/water system: metastability and equilibrium aspects. Biomaterials 21(3):223–234. https://doi.org/10.1016/S0142-9612(99)00126-X
18. Barauskas J, Landh T (2003) Phase behavior of the phytantriol/water system. Langmuir 19(23):9562–9565. https://doi.org/10.1021/la0350812
19. Cytryniak A, Żelechowska-Matysiak K, Nazaruk E, Bilewicz R, Walczak R, Majka E et al (2022) Cubosomal lipid formulation for combination cancer treatment: delivery of a chemotherapeutic agent and complexed α-particle emitter 213Bi. Mol Pharm 19(8):2818–2831. https://doi.org/10.1021/acs.molpharmaceut.2c00182
20. Alvarez-Malmagro J, Matyszewska D, Nazaruk E, Szwedziak P, Bilewicz R (2019) PM-IRRAS study on the effect of phytantriol-based cubosomes on DMPC bilayers as model lipid membranes. Langmuir 35(50):16650–16660. https://doi.org/10.1021/acs.langmuir.9b02974
21. Nazaruk E, Majkowska-Pilip A, Bilewicz R (2017) Lipidic cubic-phase nanoparticles—cubosomes for efficient drug delivery to

cancer cells. ChemPlusChem 82(4):570–575. https://doi.org/10.3390/nano10112272

22. Landau EM, Rosenbusch JP (1996) Lipidic cubic phases: a novel concept for the crystallization of membrane proteins. Proc Natl Acad Sci USA 93(25):14532–14535. https://doi.org/10.1073/pnas.93.25.14532

23. Landau EM, Rummel G, Cowan-Jacob SW, Rosenbusch JP (1997) Crystallization of a Polar Protein and Small Molecules from the Aqueous Compartment of Lipidic Cubic Phases. J Phys Chem 101(11):1935–1937. https://doi.org/10.1021/jp963347q

24. Tan C, Hosseini SF, Jafari SM (2022) Cubosomes and hexosomes as novel nanocarriers for bioactive compounds. J Agric Food Chem 70(5):1423–1437. https://doi.org/10.1021/acs.jafc.1c06747

25. Li D, Caffrey M (2020) Structure and Functional characterization of membrane integral proteins in the lipid cubic phase. J Mol Biol 432(18):5104–5123. https://doi.org/10.1016/j.jmb.2020.02.024

26. Conn CE, Drummond CJ (2013) Nanostructured bicontinuous cubic lipid self-assembly materials as matrices for protein encapsulation. Soft Matter 9(13):3449–3464. https://doi.org/10.1039/C3SM27743G

27. Caffrey M (2015) A comprehensive review of the lipid cubic phase or *in meso* method for crystallizing membrane and soluble proteins and complexes. Acta Crystallogr Sect F Struct Biol Cryst Commun 71(1):3–18. https://doi.org/10.1107/S2053230X14026843

28. Tiefenbrunn T, Liu W, Chen Y, Katritch V, Stout CD, Fee JA et al (2011) High resolution structure of the ba3 cytochrome c oxidase from *Thermus thermophilus* in a lipidic environment. PloS One 6(7):e22348. https://doi.org/10.1371/journal.pone.0022348

29. Santos JS, Asmar-Rovira GA, Han GW, Liu W, Syeda R, Cherezov V et al (2012) Crystal structure of a voltage-gated K$^+$ channel pore module in a closed state in lipid membranes. J Biol Chem 287(51):43063–43070. https://doi.org/10.1074/jbc.M112.415091

30. Zabara A, Chong JTY, Martiel I, Stark L, Cromer BA, Speziale C et al (2018) Design of ultra-swollen lipidic mesophases for the crystallization of membrane proteins with large extracellular domains. Nat Commun 9(1):1–9. https://doi.org/10.1038/s41467-018-02996-5

31. Li D, Caffrey M (2011) Lipid cubic phase as a membrane mimetic for integral membrane protein enzymes. Proc Natl Acad Sci USA 108(21):8639–8644. https://doi.org/10.1073/pnas.1101815108

32. Cherezov V, Yamashita E, Liu W, Zhalnina M, Cramer WA, Caffrey M (2006) In meso structure of the cobalamin transporter, BtuB, at 19.5 Å resolution. J Mol Biol 364(4):716–34. https://doi.org/10.1016/j.jmb.2006.09.022

33. Rasmussen SGF, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS et al (2011) Crystal structure of the β 2 adrenergic receptor–Gs protein complex. Nature 477(7366):549–555. https://doi.org/10.1038/nature10361

34. Kang Y, Gao X, Zhou XE, He Y, Melcher K, Xu HE (2016) A structural snapshot of the rhodopsin–arrestin complex. FEBS J 283(5):816–821. https://doi.org/10.1111/febs.13561

35. Sęk S, Vacek J, Dorčák V (2019) Electrochemistry of peptides. Curr Opin. Electrochem 14:166–172. https://doi.org/10.1016/j.coelec.2019.03.002

36. Vacek J, Zatloukalova M, Novak D (2018) Electrochemistry of membrane proteins and protein–lipid assemblies. Curr Opin Electrochem 12:73–80. https://doi.org/10.1016/j.coelec.2018.04.012

37. Qutub Y, Reviakine I, Maxwell C, Navarro J, Landau EM, Vekilov PG (2004) Crystallization of transmembrane proteins *in cubo*: mechanisms of crystal growth and defect formation. J

Mol Biol 343(5):1243–1254. https://doi.org/10.1016/j.jmb.2004.09.022

38. Chiu ML, Nollert P, MeC L, Belrhali H, Pebay-Peyroula E, Rosenbusch JP et al (2000) Crystallization *in cubo*: general applicability to membrane proteins. Acta Crystallogr D 56(6):781–784. https://doi.org/10.1107/s0907444900004716

39. Cherezov V (2011) Lipidic cubic phase technologies for membrane protein structural studies. Curr Opin Struct Biol 21(4):559–566. https://doi.org/10.1016/j.sbi.2011.06.007

40. Ma P, Weichert D, Aleksandrov LA, Jensen TJ, Riordan JR, Liu X et al (2017) The cubicon method for concentrating membrane proteins in the cubic mesophase. Nat Protoc 12(9):1745–1762. https://doi.org/10.1038/nprot.2017.057

41. Belrhali H, Nollert P, Royant A, Menzel C, Rosenbusch JP, Landau EM et al (1999) Protein, lipid and water organization in bacteriorhodopsin crystals: a molecular view of the purple membrane at 1.9 Å resolution. Structure 7(8):909–17. https://doi.org/10.1016/s0969-2126(99)80118-x

42. Vacek J, Zatloukalova M, Geleticova J, Kubala M, Modriansky M, Fekete L et al (2016) Electrochemical platform for the detection of transmembrane proteins reconstituted into liposomes. Anal Chem 88(8):4548–4556. https://doi.org/10.1021/acs.analchem.6b00618

43. Rowiński P, Korytkowska A, Bilewicz R (2003) Diffusion of hydrophilic probes in bicontinuous lipidic cubic phase. Chem Phys Lipids 124(2):147–156. https://doi.org/10.1016/s0009-3084(03)00051-3

44. Nazaruk E, Górecka E, Bilewicz R (2012) Enzymes and mediators hosted together in lipidic mesophases for the construction of biodevices. J Colloid Interface Sci 385(1):130–136. https://doi.org/10.1016/j.jcis.2012.06.087

45. Nazaruk E, Landau EM, Bilewicz R (2014) Membrane bound enzyme hosted in liquid crystalline cubic phase for sensing and fuel cells. Electrochim Acta 140:96–100. https://doi.org/10.1016/j.electacta.2014.05.130

46. Sun W, Vallooran JJ, Fong W-K, Mezzenga R (2016) Lyotropic liquid crystalline cubic phases as versatile host matrices for membrane-bound enzymes. J Phys Chem Lett 7(8):1507–1512. https://doi.org/10.1021/acs.jpclett.6b00416

47. Zatloukalová M, Nazaruk E, Novak D, Vacek J, Bilewicz R (2018) Lipidic liquid crystalline cubic phases for preparation of ATP-hydrolysing enzyme electrodes. Biosens Bioelectron 100:437–444. https://doi.org/10.1016/j.bios.2017.09.036

48. Speziale C, Salvati Manni L, Manatschal C, Landau EM, Mezzenga R (2016) A macroscopic H$^+$ and Cl$^-$ ions pump via reconstitution of EcClC membrane proteins in lipidic cubic mesophases. Proc Natl Acad Sci USA 113(27):7491–7496. https://doi.org/10.1073/pnas.1603965113

49. Miszta P, Nazaruk E, Nieciecka D, Możajew M, Krysiński P, Bilewicz R et al (2022) The EcCLC antiporter embedded in lipidic liquid crystalline films–molecular dynamics simulations and electrochemical methods. Phys Chem Chem Phys 24(5):3066–3077. https://doi.org/10.1039/D1CP03992J

50. Speziale C, Zabara AF, Drummond CJ, Mezzenga R (2017) Active gating, molecular pumping, and turnover determination in biomimetic lipidic cubic mesophases with reconstituted membrane proteins. ACS Nano 11(11):11687–11693. https://doi.org/10.1021/acsnano.7b06838

51. Angelova A, Ollivon M, Campitelli A, Bourgaux C (2003) Lipid cubic phases as stable nanochannel network structures for protein biochip development: X-ray diffraction study. Langmuir 19(17):6928–6935. https://doi.org/10.1021/la0345284

52. Razumas V, Kanapienien JJ, Nylander T, Engström S, Larsson K (1994) Electrochemical biosensors for glucose, lactate, urea, and creatinine based on enzymes entrapped in a cubic liquid

crystalline phase. Anal Chim Acta 289:155–162. https://doi.org/10.1016/0003-2670(94)80098-7

53. Nylander T, Mattisson C, Razumas V, Miezis Y, Håkansson B (1996) A study of entrapped enzyme stability and substrate diffusion in a monoglyceride-based cubic liquid crystalline phase. Colloids Surf 114:311–320. https://doi.org/10.1016/0927-7757(96)03563-7

54. Gao F, Yao Z, Huang Q, Chen X, Guo X, Ye Q et al (2011) A hydrogen peroxide biosensor based on the direct electron transfer of hemoglobin encapsulated in liquid-crystalline cubic phase on electrode. Colloids Surf 82(2):359–364. https://doi.org/10.1016/j.colsurfb.2010.09.009

55. Nazaruk E, Bilewicz R (2007) Catalytic activity of oxidases hosted in lipidic cubic phases on electrodes. Bioelectrochemistry 71:8–14. https://doi.org/10.1016/j.bioelechem.2006.12.007

56. Aghbolagh MS, Khani Meynaq MY, Shimizu K, Lindholm-Sethson B (2017) Aspects on mediated glucose oxidation at a supported cubic phase. Bioelectrochemistry 118:8–13. https://doi.org/10.1016/j.bioelechem.2017.06.010

57. Wu G, Yao Z, Fei B, Gao F (2017) An enzymatic ethanol biosensor and ethanol/air biofuel cell using liquid-crystalline cubic phases as hosting matrices to co-entrap enzymes and mediators. J Electrochem Soc 164(7):G82. https://doi.org/10.1149/2.0791707jes

58. Liu Y, Qu X, Guo H, Chen H, Liu B, Dong S (2006) Facile preparation of amperometric laccase biosensor with multifunction based on the matrix of carbon nanotubes–chitosan composite. Biosens Bioelectron 21(12):2195–2201. https://doi.org/10.1016/j.bios.2005.11.014

59. Grippo V, Ma S, Ludwig R, Gorton L, Bilewicz R (2019) Cellobiose dehydrogenase hosted in lipidic cubic phase to improve catalytic activity and stability. Bioelectrochemistry 125:134–141. https://doi.org/10.1016/j.bioelechem.2017.10.003

60. Ropers M-H, Bilewicz R, Stébé M-J, Hamidi A, Miclo A, Rogalska E (2001) Fluorinated and hydrogenated cubic phases as matrices for immobilisation of cholesterol oxidase on electrodes. Phys Chem Chem Phys 3(2):240–245. https://doi.org/10.1039/B007718F

61. Rowinski P, Kang C, Shin H, Heller A (2007) Mechanical and chemical protection of a wired enzyme oxygen cathode by a cubic phase lyotropic liquid crystal. Anal Chem 79(3):1173–1180. https://doi.org/10.1021/ac061325m

62. Ericsson B, Larsson K, Fontell K (1983) A cubic protein-monoolein-water phase. Biochim Biophys Acta 729(1):23–27. https://doi.org/10.1016/0005-2736(83)90451-0

63. Razumas V, Talaikytė Z, Barauskas J, Miezis Y, Nylander T (1997) An FT-IR study of the effects of distearoylphosphatidylglycerol and cytochrome c on the molecular organization of the monoolein-water cubic liquid-crystalline phase. Vib Spectrosc 15(1):91–101. https://doi.org/10.1016/S0924-2031(97)00021-0

64. Razumas V, Larsson K, Miezis Y, Nylander T (1996) A cubic monoolein– cytochrome c– water phase: X-ray diffraction, ft-ir, differential scanning calorimetric, and electrochemical studies. J Phys Chem A 100(28):11766–11774. https://doi.org/10.1021/jp952613h

65. Kraineva J, Narayanan RA, Kondrashkina E, Thiyagarajan P, Winter R (2005) Kinetics of lamellar-to-cubic and intercubic phase transitions of pure and cytochrome c containing monoolein dispersions monitored by time-resolved small-angle X-ray diffraction. Langmuir 21(8):3559–3571. https://doi.org/10.1021/la046873e

66. Barauskas J, Razumas V, Talaikyt Z, Bulovas A, Nylander T, Taurait D et al (2003) Towards redox active liquid crystalline phases of lipids: a monoolein/water system with entrapped derivatives of ferrocene. Chem Phys Lipids 123(1):87–97. https://doi.org/10.1016/S0009-3084(02)00170-6

67. Kostela J, Elmgren M, Kadi M, Almgren M (2005) Redox activity and diffusion of hydrophilic, hydrophobic, and amphiphilic redox active molecules in a bicontinuous cubic phase. J Phys Chem B 109(11):5073–5078. https://doi.org/10.1021/jp048088g

68. Kumar PS, Lakshminarayanan V (2007) Electron-transfer studies in a lyotropic columnar hexagonal liquid crystalline medium. Langmuir 23(3):1548–1554. https://doi.org/10.1021/la0625244

69. Nazaruk E, Miszta P, Filipek S, Gorecka E, Landau EM, Bilewicz R (2015) Lyotropic cubic phases for drug delivery: diffusion and sustained release from the mesophase evaluated by electrochemical methods. Langmuir 31(46):12753–12761. https://doi.org/10.1021/acs.langmuir.5b03247

70. Antognini LM, Assenza S, Speziale C, Mezzenga R (2016) Quantifying the transport properties of lipid mesophases by theoretical modelling of diffusion experiments. J Chem Phys 145(8):084903. https://doi.org/10.1063/1.4961224

71. Esposito E, Ravani L, Mariani P, Contado C, Drechsler M, Puglia C et al (2013) Curcumin containing monoolein aqueous dispersions: a preformulative study. Mater Sci Eng C 33(8):4923–4934. https://doi.org/10.1016/j.msec.2013.08.017

72. Linkevičiūtė A, Misiūnas A, Naujalis E, Barauskas J (2015) Preparation and characterization of quercetin-loaded lipid liquid crystalline systems. Colloids Surf B 128:296–303. https://doi.org/10.1016/j.colsurfb.2015.02.001

73. Yaghmur A, Mu H (2021) Recent advances in drug delivery applications of cubosomes, hexosomes, and solid lipid nanoparticles. Acta Pharm Sin B 11:871–885. https://doi.org/10.1016/j.apsb.2021.02.013

74. Larsson K (1989) Cubic lipid-water phases: structures and biomembrane aspects. J Phys Chem 93(21):7304–7314. https://doi.org/10.1021/j100358a010

75. Karami Z, Hamidi M (2016) Cubosomes: remarkable drug delivery potential. Drug Discov Today 21(5):789–801. https://doi.org/10.1016/j.drudis.2016.01.004

76. Spicer PT, Hayden KL, Lynch ML, Ofori-Boateng A, Burns JL (2001) Novel process for producing cubic liquid crystalline nanoparticles (cubosomes). Langmuir 17(19):5748–5756. https://doi.org/10.1021/la010161w

77. Mezzenga R, Meyer C, Servais C, Romoscanu AI, Sagalowicz L, Hayward RC (2005) Shear rheology of lyotropic liquid crystals: a case study. Langmuir 21(8):3322–3333. https://doi.org/10.1021/la046964b

78. Hirlekar R, Jain S, Patel M, Garse H, Kadam V (2010) Hexosomes: a novel drug delivery system. Curr Drug Deliv 7(1):28–35. https://doi.org/10.2174/156720110790396526

79. Angelova A, Garamus VM, Angelov B, Tian Z, Li Y, Zou A (2017) Advances in structural design of lipid-based nanoparticle carriers for delivery of macromolecular drugs, phytochemicals and anti-tumor agents. Adv Colloid Interface Sci 249:331–345. https://doi.org/10.1016/j.cis.2017.04.006

80. Murgia S, Biffi S, Mezzenga R (2020) Recent advances of non-lamellar lyotropic liquid crystalline nanoparticles in nanomedicine. Curr Opin Colloid Interface Sci 48:28–39. https://doi.org/10.1016/j.cocis.2020.03.006

81. Tenchov R, Bird R, Curtze AE, Zhou Q (2021) Lipid Nanoparticles─ From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement. ACS Nano 15(11):16982–17015. https://doi.org/10.1021/acsnano.1c04996

82. Tilley AJ, Drummond CJ, Boyd BJ (2013) Disposition and association of the steric stabilizer Pluronic® F127 in lyotropic liquid crystalline nanostructured particle dispersions. J Colloid Interface Sci 392:288–296. https://doi.org/10.1016/j.jcis.2012.09.051

83. Murgia S, Falchi AM, Mano M, Lampis S, Angius R, Carnerup AM et al (2010) Nanoparticles from lipid-based liquid crystals: emulsifier influence on morphology and cytotoxicity. J Phys

Chem B 114(10):3518–3525. https://doi.org/10.1021/jp9098655

84. Chong JY, Mulet X, Waddington LJ, Boyd BJ, Drummond CJ (2011) Steric stabilisation of self-assembled cubic lyotropic liquid crystalline nanoparticles: high throughput evaluation of triblock polyethylene oxide-polypropylene oxide-polyethylene oxide copolymers. Soft Matter 7(10):4768–4777. https://doi.org/10.1039/C1SM05181D

85. Yaghmur A, Glatter O (2009) Characterization and potential applications of nanostructured aqueous dispersions. Adv Colloid Interface Sci 147:333–342. https://doi.org/10.1016/j.cis.2008.07.007

86. Zhai J, Waddington L, Wooster TJ, Aguilar M-I, Boyd BJ (2011) Revisiting β-Casein as a stabilizer for lipid liquid crystalline nanostructured particles. Langmuir 27(24):14757–14766. https://doi.org/10.1021/la203061f

87. Nilsson C, Østergaard J, Larsen SW, Larsen C, Urtti A, Yaghmur A (2014) PEGylation of phytantriol-based lyotropic liquid crystalline particles the effect of lipid composition, PEG chain length, and temperature on the internal nanostructure. Langmuir 30(22):6398–6407. https://doi.org/10.1021/la501411w

88. Angelova A, Angelov B, Garamus VM, Couvreur P, Lesieur S (2012) Small-angle X-ray scattering investigations of biomolecular confinement, loading, and release from liquid-crystalline nanochannel assemblies. J Phys Chem Lett 3(3):445–457. https://doi.org/10.1021/jz2014727

89. Helvig S, Azmi ID, Moghimi SM, Yaghmur A (2015) Recent advances in cryo-TEM imaging of soft lipid nanoparticles. AIMS Biophys 2(2):116–130. https://doi.org/10.3934/biophy.2015.2.116

90. Neto C, Aloisi G, Baglioni P, Larsson K (1999) Imaging soft matter with the atomic force microscope: cubosomes and hexosomes. J Phys Chem B 103(19):3896–3899. https://doi.org/10.1021/jp984551b

91. Fraser SJ, Mulet X, Martin L, Praporski S, Mechler A, Hartley PG et al (2012) Surface immobilization of bio-functionalized cubosomes: sensing of proteins by quartz crystal microbalance. Langmuir 28(1):620–627. https://doi.org/10.1021/la2032994

92. Barriga HM, Holme MN, Stevens MM (2019) Cubosomes: the next generation of smart lipid nanoparticles? Angew Chem Int Ed 58(10):2958–2978. https://doi.org/10.1002/anie.201804067

93. Ryan S, Shortall K, Dully M, Djehedar A, Murray D, Butler J et al (2022) Long acting injectables for therapeutic proteins. Colloid Surf B-Biointerfaces 217:112644. https://doi.org/10.1016/j.colsurfb.2022.112644

94. Nazaruk E, Szlęzak M, Górecka E, Bilewicz R, Osornio YM, Uebelhart P et al (2014) Design and assembly of pH-sensitive lipidic cubic phase matrices for drug release. Langmuir 30(5):1383–1390. https://doi.org/10.1021/la403694e

95. Falchi AM, Rosa A, Atzeri A, Incani A, Lampis S, Meli V et al (2015) Effects of monoolein-based cubosome formulations on lipid droplets and mitochondria of HeLa cells. Toxicol Res 4(4):1025–1036. https://doi.org/10.1039/C5TX00078E

96. Tudose A, Celia C, Belu I, Borisova S, Paolino D (2014) Effect of three monoglyceride based cubosomes systems on the viability of human keratinocytes. Farmacia 62(4):777–790

97. Caltagirone C, Arca M, Falchi AM, Lippolis V, Meli V, Monduzzi M et al (2015) Solvatochromic fluorescent BODIPY derivative as imaging agent in camptothecin loaded hexosomes for possible theranostic applications. RSC Adv 5(30):23443–23449. https://doi.org/10.1039/C5RA01025J

98. Cytryniak A, Nazaruk E, Bilewicz R, Górzyńska E, Żelechowska-Matysiak K, Walczak R et al (2020) Lipidic cubic-phase nanoparticles (cubosomes) loaded with doxorubicin and labeled with 177Lu as a potential tool for combined chemo and internal radiotherapy for cancers. Nanomaterials 10(11):2272. https://doi.org/10.3390/nano10112272

99. Mat Azmi ID, Wu L, Wibroe PP, Nilsson C, Østergaard J, Sturup S et al (2015) Modulatory effect of human plasma on the internal nanostructure and size characteristics of liquid-crystalline nano-carriers. Langmuir 31(18):5042–5049. https://doi.org/10.1021/acs.langmuir.5b00830

100. Nielsen LS, Schubert L, Hansen J (1998) Bioadhesive drug delivery systems: I. characterisation of mucoadhesive properties of systems based on glyceryl mono-oleate and glyceryl monolinoleate. Eur J Pharm Sci 6(3):231–9. https://doi.org/10.1016/s0928-0987(97)10004-5

101. Souza C, Watanabe E, Borgheti-Cardoso LN, Fantini MCDA, Lara MG (2014) Mucoadhesive system formed by liquid crystals for buccal administration of poly (hexamethylene biguanide) hydrochloride. J Pharm Sci 103(12):3914–3923. https://doi.org/10.1002/jps.24198

102. Dully M, Brasnett C, Djeghader A, Seddon A, Neilan J, Murray D et al (2020) Modulating the release of pharmaceuticals from lipid cubic phases using a lipase inhibitor. J Colloid Interface Sci 573:176–192. https://doi.org/10.1016/j.jcis.2020.04.015

103. Zatloukalová M, Jedinák L, Riman D, Franková J, Novák D, Cytryniak A et al (2021) Cubosomal lipid formulation of nitroalkene fatty acids: preparation, stability and biological effects. Redox Biol 46:102097. https://doi.org/10.1016/j.redox.2021.102097

104. Akbar S, Anwar A, Ayish A, Elliott JM, Squires AM (2017) Phytantriol based smart nano-carriers for drug delivery applications. Eur J Pharm Sci 101:31–42. https://doi.org/10.1016/j.ejps.2017.01.035

105. Sobczak K, Rudnicki K, Jedinak L, Zatloukalova M, Vacek J, Poltorak L (2022) Oleic and nitro-oleic acid behavior at an electrified water-1, 2-dichloroethane interface. J Mol Liq 365:120110. https://doi.org/10.1016/j.molliq.2022.120110

106. Laborda E, Molina A, Espín VF, Martínez-Ortiz F, Garcia de la Torre J, Compton RG (2017) Single fusion events at polarized liquid–liquid interfaces. Angew Chem Int Ed 129(3):800–803. https://doi.org/10.1002/anie.201610185

107. Laborda E, Molina A (2021) Impact experiments at the interface between two immiscible electrolyte solutions (ITIES). Curr Opin Electrochem 26:100664. https://doi.org/10.1016/j.coelec.2020.100664

108. Trojánek A, Samec Z (2019) Study of the emulsion droplet collisions with the polarizable water/1, 2-dichloroethane interface by the open circuit potential measurements. Electrochim Acta 299:875–885. https://doi.org/10.1016/j.electacta.2019.01.041

109. Trojánek A, Mareček V, Samec Z (2018) Open circuit potential transients associated with single emulsion droplet collisions at an interface between two immiscible electrolyte solutions. Electrochem Commun 86:113–116. https://doi.org/10.1016/j.elecom.2017.11.026

110. Santos HA, García-Morales V, Pereira CM (2010) Electrochemical properties of phospholipid monolayers at liquid–liquid interfaces. ChemPhysChem 11(1):28–41. https://doi.org/10.1002/cphc.200900609