



Stimuli-responsive lyotropic liquid crystalline nanoparticles: Adaptive soft matter in action

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Lyotropic liquid crystalline nanoparticles (LLCNPs) such as cubosomes and hexosomes have emerged as versatile nanocarriers for the delivery of bioactive agents due to their tunable internal structures, high surface area, and ability to encapsulate hydrophilic, hydrophobic, and amphiphilic cargos. Lipid nanoparticles self-assembled from amphiphilic lipids, such as monoolein and phytantriol, or their polymer analogs, form ordered mesophases whose curvature and lattice parameters can be modulated through composition. The unique internal nanostructures of LLCNPs critically influence their physicochemical properties, which in turn affect cellular uptake mechanisms and drug release profiles. Stimuli-responsiveness – pH, enzymatic activity, temperature, ionic strength, magnetic field, oxidation, and light – can be engineered by incorporating ionizable lipids, phospholipids, responsive polymers, or photosensitive molecules, enabling controlled and on-demand release. Moreover, hybrid systems incorporating magnetic or polymeric components further expand LLCNPs functionality. This review provides a comprehensive overview of the design principles and stimuli-responsive behavior of cubosomes and hexosomes, emphasizing their potential for precision drug delivery.

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Introduction

Lyotropic liquid crystalline nanoparticles (LLCNPs), particularly cubosomes and hexosomes, represent an advanced and rapidly evolving class of self-assembled lipid-based nanocarriers characterized by highly ordered and tunable internal nanostructures [1,2]. These systems originate mainly from the dispersion of inverse lyotropic liquid crystalline phases formed by amphiphilic lipids, mostly monoolein (MO) and phytantriol (PT) in excess aqueous environments. Remarkably, this research also stimulated interest in their polymer analogs (polymer cubosomes or polymer hexosomes) [3]. Depending on lipid composition, hydration level, and environmental conditions, these materials self-organize into bicontinuous cubic phases (V_2 , cubosomes) or inverse hexagonal phases (H_2 , hexosomes). The structural organization of the V_2 phase is intimately related to infinite periodic minimal surfaces (IPMS), such as the double gyroid (Ia3d), double diamond (Pn3m), and primitive (Im3m) symmetries. These mathematically defined surfaces exhibit zero mean curvature and divide space into two interpenetrating aqueous channel networks without intersection. In contrast, hexosomes are composed of cylindrical inverse micelles arranged in a hexagonal lattice [4–6]. These topologies provide cubosomes and hexosomes with a remarkably high internal surface area and continuous diffusion pathways, which are key features for sustained and controlled drug release [7]. The ability to access and control these distinct nanostructures could be central to the design of LLCNPs for specific biomedical applications (Table 1).

A fundamental concept governing the self-assembly and phase behavior of these lipid systems is the critical packing parameter (CPP), defined as $CPP = v/(a_0 l_c)$, where v is the hydrophobic chain volume, a_0 is the effective headgroup area, and l_c is the chain length. The CPP provides a predictive framework for understanding how molecular geometry dictates the curvature of lipid assemblies, as exemplified in Figure 1. Lipids with $CPP \approx 1$ tend to form lamellar phases, whereas those with $CPP > 1$ favor inverse structures, characterized by

Table 1

Stimuli-responsive lyotropic liquid crystalline nanoparticles.

Stimulus	Structural response	Responsive component	Payload	Evidence	Ref.
Enzyme	$V_2 \rightarrow H_2, I_2, L_3$	Monoolein	Not investigated	S	[22]
Enzyme	$V_2 \rightarrow H_2$	Triglycerides	Not investigated	S	[23]
Enzyme	$L_\alpha \rightarrow V_2$	Sucrose laurate	Fluorescein	S + R	[24]
Enzyme	$V_2 \rightarrow H_2$	Tween 80	Not investigated	S	[25]
Enzyme	mesophase modulation (I_2/H_2)	Oat oil polar lipids	Curcumin, vitamin D, octyl glucoside	S	[26]
Enzyme	V_2 (Pn3m) $\rightarrow V_2$ (Im3m)	Phospholipids	Not investigated	S	[27]
Enzyme	Phase/morphology changes	Cyclopropanated/cis-unsaturated lipids	Not investigated	S	[28]
H_2O_2	PV_2 morphological transition/disassembly	PEO-b-PFEMA	Not investigated	S	[29]
H_2O_2	PV_2/PH_2 morphological transition/disassembly	Oxidation-sensitive polymer blocks	Not investigated	S	[30]
H_2O_2	PV_2 disassembly	Boronic ester-functionalized block	Not investigated	S	[31]
Ionic strength	$L_\alpha \rightarrow V_2$	Ionic surfactants (DDAB/AOT)	Not investigated	S	[32]
Ionic strength	$H_2 \leftrightarrow V_2 \leftrightarrow L_\alpha/L_3$ phase transitions	Protic ionic liquids	Not investigated	S	[33]
Light	$L_\alpha \leftrightarrow V_2$	donor-acceptor Stenhouse adduct	FITC-dextran	S + R	[37]
Light	$V_2 \rightarrow H_2; L_\alpha \rightarrow V_2$	HABI	Not investigated	S	[38]
Light	No structural response, photothermal therapy	Indocyanine green	Doxorubicine	S + R + C + V	[39]
Light	Contraction of the V_2 lattice	Azobenzene-based photosurfactants	Nile red	S + R	[40]
Light	V_2 nanostructure breakdown	PEG45-b-PNBMAm	Not investigated	S	[19]
Light	No structural response, photodynamic therapy	Chlorin e6 or mTPP Mn(III) chloride	Not investigated	S + C	[41]
Light	No structural response, photodynamic therapy	PF108-(DPP-ZnP) ₂	Docetaxel	S + C	[42]
Light	No structural response, photodynamic therapy	Up-converting nanoparticles	Daunorubicin	S + C	[43]
Light	No structural response, photodynamic therapy	Ru(II) photosensitizer	Not investigated	S + C	[44]
Light	No structural response, photodynamic therapy	Ru(II) photosensitizer	Not investigated	S + C	[45]
Light	No structural response, photodynamic therapy	Algal photosensitizer	Not investigated	S + C	[46]
AMF	Bilayer perturbation	Fe ₃ O ₄ SPIONs	Rhodamines	S + R	[48]
AMF	$V_2 \rightarrow H_2$	Hydrophobic SPIONs	Not investigated	S	[49]
AMF	$V_2 \rightarrow H_2$	Hydrophobic SPIONs	C18-Rhodamine	S + C	[50]
MF	$V_2 \rightarrow H_2$	Hydrophilic Ni-Zn ferrite NPs	B conjugated	S + R	[51]
AMF	Proposed mechanical disruption	Hydrophobic Fe ₃ O ₄ NPs	Doxorubicin	S + R	[52]
AMF	$V_2 \leftrightarrow H_2$	SPIONs	Methotrexate	S + R	[53]
AMF	$L_\alpha \rightarrow V_2$	SPIONs	Fluorescein Na	S + R	[54]
AMF	Magnetic response not investigated	SPIONs	Sulfo-Cy5	S + R	[55]
pH	No structural response, pH-triggered release	SPIONs	Helenalin	S + C	[55]
pH/temp.	$I_2 \rightarrow$ emulsion	Lipid matrix	Doxorubicin	R + C	[56]
pH	$V_2 \rightarrow$ inverse micellar/vesicular phases; lattice-spacing changes	Unsaturated acids	Not investigated	S	[57]
pH	Emulsion $\rightarrow I_2/H_2 \rightarrow$ vesicles	Nicergoline	Nicergoline	S	[58]
pH	$H_2 \rightarrow V_2 \rightarrow$ vesicles	Oleic acid	LL-37	S	[59]
pH	$H_2 \rightarrow V_2 \rightarrow$ vesicles	2OHOA	2OHOA	S	[60]
pH	$H_2 \rightarrow V_2 \rightarrow$ emulsion	Oleic acid	<i>Brucea javanica</i> oil + Doxorubicin	S + R + C	[61]
pH	$H_2 \rightarrow V_2$	Ionizable lipid O2ME	Fluconazole	S + C	[62]
pH	pH-triggered particle disruption	PP50	Not investigated	S	[63]
pH	Charge conversion; no structural response	PDMAEMA-b-PLMA	Not investigated	S	[64]
pH	Morphological changes	PDMAEMA-b-PLMA	Not investigated	S	[65]
pH	Morphological/structural rearrangements	TPP-QPDMAEMA-b-PLMA	Resveratrol	S + R + C	[66]

pH	ζ -potential conversion	TPP-QPDEMAEMA-b-PLMA	Not investigated	S	[67]
pH/H ₂ O ₂	V ₂ → L ₂ /H ₂ -L ₂ → V ₂	PDMAEMA/PTBA-containing ABC	Not investigated	S	[68]
pH/H ₂ O ₂	pH- and H ₂ O ₂ -induced mesophase transitions	PDMAEMA/PTBA blocks	Paclitaxel	S + R + C	[69]
pH	V ₂ (Im3m) → V ₂ (Pn3m) transition; lattice swelling	CHarg/alginate biopolymers	Not investigated	S	[70]
pH/serum albumin	V ₂ (Im3m) → V ₂ (Pn3m) transition; lattice shrinking	CHarg/alginate biopolymers	Not investigated	S	[71]
pH	Im3m → H ₂ ; disassembly	CHarg/alginate biopolymers	Aloe vera-derived acemannan	S	[72]
pH	No structural response reported; triggered release	Sodium alginate coating	Fluorescein + Nile Red	S + R + C	[73]
pH	L ₂ → H ₂ → V ₂	Ionizable aminolipids	Not investigated	S	[74]
pH	H ₂ → V ₂	Ionizable aminolipids	SN-38	S + R	[75]
pH	L ₂ → H ₂	Vaccenic acid/vaccenyl acetate	Not investigated	S	[76]
pH	L ₂ → H ₂ → V ₂	Ionizable aminolipids (ALC-0315/SM-102)	Not investigated	S	[77]
pH	V ₂ → H ₂ /L ₂	PDMAEMA/DEAEMA copolymers	Not investigated	S	[78]
pH	V ₂ → H ₂	MO + ionizable lipid	mRNA	S + C + V	[79]
pH	V ₂ → L ₃ → vesicles	Rhamnolipids	Antimicrobial	S + C	[80]
pH	PV ₂ dissolution	P4VP	Organic dyes; Pt nanoparticles	S	[81]
pH	L ₂ → V ₂	2OHOA	Not investigated	S	[82]
pH	Q ₂ → lattice shrinking	ω -3 PUFAs (DHA/EPA)	Ginkgolide B + quercetin	S + R + C	[83]
Temperature	Order-disorder transition	PDMA side chains	Not investigated	S	[84]
Temperature	Reversible corona collapse and mesophase changes	PNIPAM	Not investigated	S	[85]

The table summarizes the nature of the stimulus, the resulting structural or morphological response, the responsive component, and the encapsulated or delivered payload. The 'Evidence' column indicates the level of experimental evidence supporting the responsiveness of each system according to the following legend: Structural characterization only = S; Stimulus-dependent cargo release = R; Cellular/in vitro evidence = C; In vivo evidence = V. PV₂ = polymer cubosomes. AMF, alternating magnetic field; NPs, nanoparticles; SPIONs, superparamagnetic iron oxide nanoparticles.

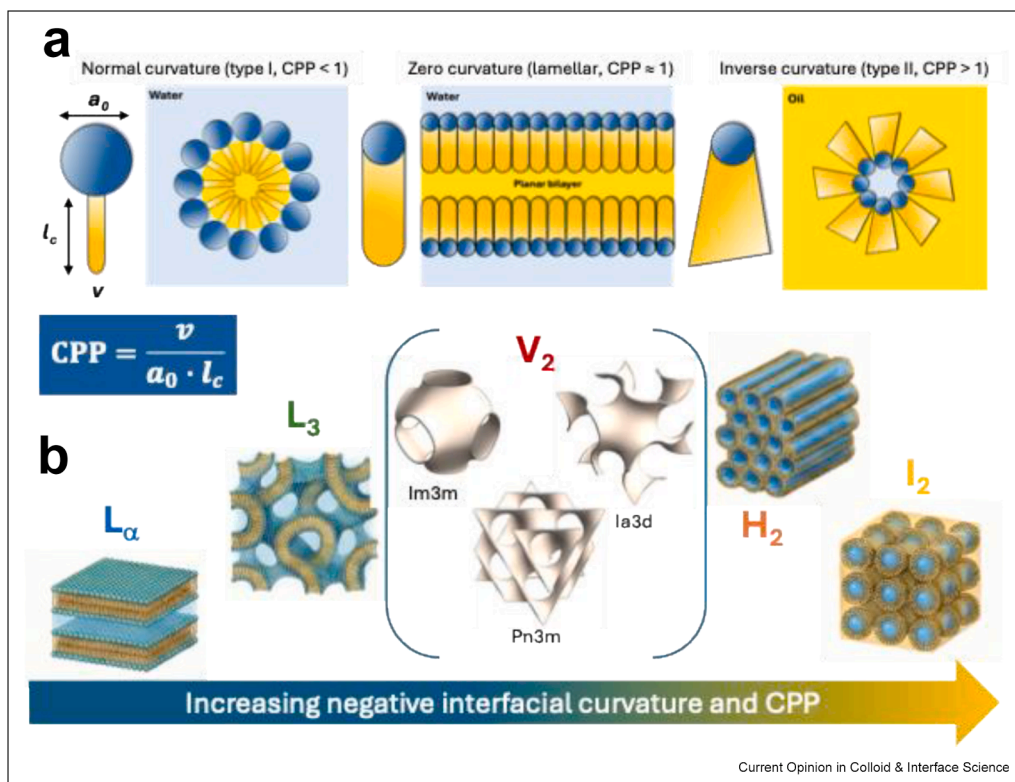
negative interfacial curvature (i.e., bending away from the aqueous phase, see Figure 1) such as cubic and hexagonal phases. Importantly, environmental factors such as pH, ionic strength, and temperature can modify a_0 and/or v , thereby altering the CPP and inducing phase transitions. This principle underlies many of the stimuli-responsive behaviors observed in LLCNP systems.

Although cubosomes and hexosomes were first identified several decades ago, their pharmaceutical relevance has significantly expanded only in recent years. Their structural versatility, high internal surface area, and capacity to host diverse payloads make them highly attractive as nanocarriers for drug delivery [8], gene therapy, and imaging applications. A major advancement in this field is the development of stimuli-responsive LLCNPs, capable of undergoing structural transformations in response to environmental triggers such as pH, temperature, ionic strength, oxidative stress, and, when photoresponsive components are incorporated, light. In parallel, light activation of encapsulated photosensitizers (PSs) can trigger the generation of reactive oxygen species (ROS), enabling photodynamic therapy. These approaches allow precise tuning of ROS production and structural transitions and drug release profiles, on demand or in response to specific pathological conditions [9,10].

The physicochemical properties of LLCNPs are strongly influenced by lipid composition. MO is widely employed due to its ability to form more biocompatible and stable bicontinuous cubic phases in excess water, while PT offers enhanced chemical stability and resistance to hydrolysis. The inclusion of additional components such as phospholipids or charged amphiphiles can further modulate membrane curvature, mechanical properties, and responsiveness. These compositional variables enable fine control over mesophase behavior and stability, as well as interactions with biological environments [11]. Polymer cubosomes, on the other hand, offer complementary advantages, including virtually unlimited possibilities for molecular design of stimuli-responsive and functional architectures, as well as greater flexibility in tuning pore size over a wider range. Examples of lipid- and polymer-based cubosomes and hexosomes are given in Figure 2.

From a colloidal standpoint, LLCNPs typically exhibit hydrodynamic diameters in the range of 100–300 nm, low polydispersity, and surface charges that depend on composition and stabilizing agents. These nanoparticles require stabilization in aqueous dispersions to prevent aggregation. This is commonly achieved using steric stabilizers such as Pluronic F127 (PF127), Pluronic F108 (PF108), or other amphiphilic block

Figure 1



(a) Schematic illustration of surfactant molecular geometry and the resulting aggregate morphologies as a function of interfacial curvature, including type I, lamellar (zero curvature), and type II structures. (b) Schematic evolution of lyotropic liquid crystalline phases in lipid–water systems as a function of increasing negative interfacial curvature. The sequence includes lamellar (L_2), sponge (L_3), bicontinuous cubic (V_2 , also reported are the IPMS symmetries for the primitive, Im3m, double diamond, Pn3m, and double gyroid, Ia3d, phases), hexagonal (H_2), and discontinuous micellar cubic (I_2 , Fd3m or Fm3m symmetry) phases. IPMS, infinite periodic minimal surfaces.

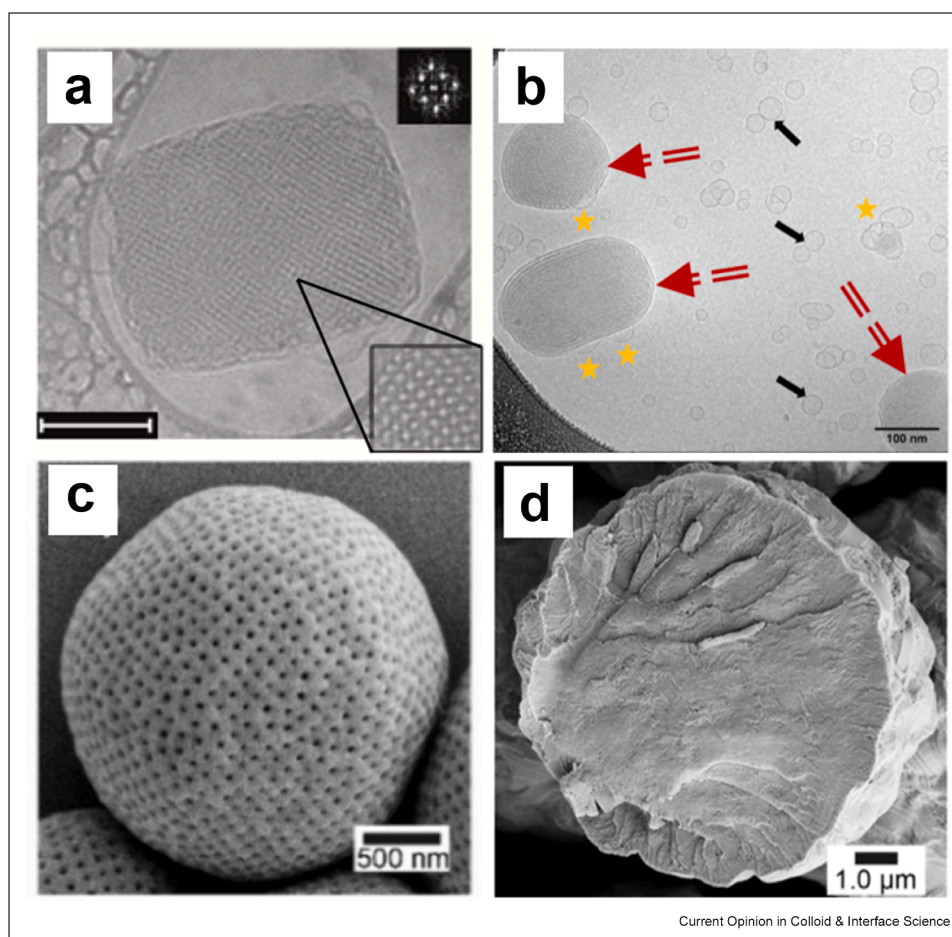
copolymers, which adsorb at the particle surface and provide steric repulsion. More advanced stabilizers can also introduce functional properties, including targeting ligands or additional responsiveness to environmental stimuli.

Various preparation methods have been developed to produce LLCNPs, broadly classified into top-down and bottom-up approaches. Top-down methods involve the fragmentation of bulk liquid crystalline phases using high-energy techniques such as high-pressure homogenization or ultrasonication. In contrast, bottom-up approaches rely on spontaneous self-assembly processes, often involving solvent exchange, dilution, or hydrotrope-mediated techniques, possibly using microfluidic platforms [12,13]. These methods can offer improved control over particle size and internal structure but may require careful optimization of processing conditions. The choice of preparation method significantly influences the final properties of the nanoparticles, including size distribution and stability. Comprehensive characterization of LLCNPs requires a

combination of complementary analytical techniques. Small-angle X-ray scattering (SAXS) is the primary tool for determining mesophase symmetry, lattice parameters, and structural transitions. Cryogenic transmission electron microscopy (cryo-TEM) provides direct visualization of nanoparticle morphology and internal nanostructure. Dynamic light scattering (DLS) is widely used to measure particle size and polydispersity, while zeta potential analysis provides insight into surface charge and colloidal stability. More innovative techniques, such as a combination of correlative cryogenic 3D fluorescence (Cryo-SIM) and cryogenic soft X-ray tomography (Cryo-SXT), allow visualization of the intracellular localization of these nanoparticles [14].

Beyond their physicochemical properties, and considering their predicted applications in the pharmaceutical field, the interaction of LLCNPs with biological systems is a critical determinant of their performance as drug delivery platforms [15]. Particularly, a recent study has demonstrated that the internal nanostructure plays a key role in governing cellular uptake pathways [16].

Figure 2



Cryo-TEM micrographs of lipid-based cubosome (a) scale bar is 100 nm, reprinted from Ref. [17] with permission from Elsevier) and hexosomes (b indicated by red arrows, reprinted (adapted) with permission from Ref. [18]. Copyright 2025 American Chemical Society). SEM micrographs in c and d, respectively, represent polymer-based cubosomes (c) and hexosomes (d), both reprinted (adapted) with permission from Ref. [19]. Copyright 2024 American Chemical Society. cryo-TEM, cryogenic transmission electron microscopy; SEM, scanning electron microscopy.

LLCNPs generally exhibit enhanced cellular internalization compared to lamellar liposomes. This behavior is attributed to their ability to engage both energy-dependent endocytic pathways and passive mechanisms such as membrane fusion. Macropinocytosis has been identified as a dominant endocytic route for nanoparticles of typical sizes (~ 200 nm), while membrane fusion may facilitate direct cytosolic delivery by bypassing endosomal entrapment. Importantly, it has been shown that the type of internal mesophase can influence cellular uptake kinetics/modalities, independently of particle size, surface charge, and stabilizer. In this study [16], cubosomes consistently demonstrated higher uptake levels compared to hexosomes and liposomes, highlighting the possible role of membrane

curvature and internal topology in modulating nanoparticle–cell interactions. While differences among non-lamellar phases are relatively subtle, the distinction between lamellar and non-lamellar structures appears to be a key determinant of uptake efficiency and intracellular trafficking.

Overall, tunable lipid composition and advanced preparation and characterization techniques have established LLCNPs as a versatile and powerful platform for nanomedicine. Their ability to combine structural complexity with stimuli-responsive behavior and favorable biological interactions makes cubosomes and hexosomes particularly promising for next-generation drug delivery systems.

Stimuli-responsive lyotropic liquid crystalline nanoparticles

Enzyme-responsive

Enzymes represent highly selective biological triggers that can be exploited to design adaptive nanomaterials capable of responding to biochemical cues present in specific physiological environments. Enzyme-responsive nanocarriers typically rely on enzymatic reactions that modify the chemical structure of their building blocks, thereby inducing degradation, bond cleavage, or structural reorganization of the nanomaterial. As highlighted in several recent reviews [20,21], these systems take advantage of the catalytic activity and substrate specificity of enzymes to achieve controlled and localized activation of drug delivery systems. Enzyme-responsive nanoparticles have been widely investigated for applications in chemotherapy, inflammation-related diseases, and targeted drug delivery, where enzymes such as proteases, lipases, and phospholipases are often overexpressed in pathological tissues. Within this context, LLCNPs such as cubosomes, provide a particularly interesting platform for enzyme-responsive nanomaterials.

Well-ordered glycerol MO-based V_2 LLCNPs serve as model substrates to study lipase-catalyzed hydrolysis, linking enzymatic activity to nanostructural transformations. Cryo-TEM and synchrotron SAXS reveal that pH-dependent phase transitions – from V_2 to H_2 , I_2 , and L_3 -like structures – modulate the lipolysis rate, influenced by particle size and surface defects. These findings demonstrate how the internal organization of lipid nanoparticles, combined with environmental factors like pH, governs enzyme activity, offering versatile tools for industrial, pharmaceutical, and biotechnological applications [22].

Incorporating digestion-sensitive triglycerides into PT-based LLCNPs enables dynamic, stimuli-responsive behavior driven by enzymatic cleavage and local pH. Bulky triglycerides are hydrolyzed into smaller fatty acids (FAs), which intercalate within the lipid bilayer, modulating the CPP and inducing transitions between V_2 (Pn3m) and H_2 mesophases. Protonation under acidic conditions favors H_2 formation with minimal drug release, whereas ionization at higher pH stabilizes cubic phases, enhancing mucoadhesion, and compound release. This enzymatic and pH-triggered adaptability offers a versatile strategy for oral drug delivery, allowing the in situ formation of functional nanostructures and controlled release kinetics [23]. Similarly, including sucrose laurate in PT or monolinolein lipid bilayers generates vesicles that respond to specific enzymatic triggers. Upon exposure to invertase, the sucrose head-group is hydrolyzed, increasing the hydrophobicity of the molecule and inducing a dynamic transformation from vesicles to cubosomes (Im3m phase). This structural rearrangement enables the controlled release of

encapsulated compounds. By combining PT and monolinolein, dual responsiveness to both invertase and lipase can be achieved [24].

A strategy to develop enzyme-responsive LLCNPs exploited fungal lipase activity by designing systems in which only a minor component is enzymatically cleavable [25]. In this formulation, PT formed stable cubosomes with a bicontinuous cubic phase, while Tween 80 acted both as a stabilizer and a lipase substrate. Upon enzymatic hydrolysis, Tween 80 generated oleic acid, a lipid favoring higher negative curvature, thus promoting phase transitions toward more curved mesophases. The response was tested using two fungal lipases: *Candida antarctica* lipase B (CALB) and *Rhizomucor miehei* lipase (RMML). CALB efficiently hydrolyzed Tween 80, inducing a clear V_2 -to- H_2 phase transition, driven by oleic acid accumulation. In contrast, RMML initially produced no structural changes, likely due to limited access to the substrate at the nanoparticle interface. Importantly, incorporation of a small amount of cationic surfactant enhanced RMML activity, restoring the phase transition. This highlights the critical role of enzyme-nanoparticle interfacial interactions.

Incorporating enzymatic processing, natural oat oils enriched with polar lipids can be transformed into liquid crystalline phases with L_α , I_2 , or H_2 internal structures. The polar lipid content and lipolytic enzyme conditions dictate the curvature of the lipid–aqueous interface, enabling precise control of phase formation. Functional additives such as curcumin, vitamin D, and octyl glucoside can be incorporated without compromising stability. The resulting bulk phases can be dispersed into nanoparticles, offering tunable, biocompatible systems for controlled delivery of bioactive molecules in nutraceutical, pharmaceutical, and food applications [26]. This work highlights enzymatic processing as a promising strategy for controlling lipid–liquid-crystalline structure, enabling the formation of I_2 (Fd3m) and H_2 phases in bulk and dispersed systems.

An alternative strategy for enzyme-responsive LLCNPs exploits the susceptibility of phospholipids to hydrolysis by phospholipase A2 (PLA2) [27]. In this approach, small amounts (0–10 mol%) of phosphatidylcholines are incorporated into MO-based cubosomes to introduce enzymatic sensitivity. PLA2, which is overexpressed in several pathological conditions, catalyzes the cleavage of phospholipids into lysophospholipids and free FAs, generating amphiphilic products that accumulate at the lipid–water interface and modify membrane curvature. Upon enzymatic digestion, significant structural changes occur within the cubosomes. Specifically, a progressive swelling of the bicontinuous cubic phase is observed, followed by transitions from the Pn3m to the Im3m bicontinuous cubic structure. The extent of these

transformations depends on both the concentration and chemical nature of the incorporated phospholipids, with shorter or more unsaturated chains inducing stronger effects. Additionally, calcium ions, required for PLA2 activity, modulate the response, enhancing structural changes up to an optimal concentration before electrostatic screening effects reduce the impact. This strategy highlights how controlled phospholipid incorporation enables the design of PLA2-responsive cubosomes with potential applications for targeted drug delivery.

Hypothesizing that incorporating subtle modifications in the molecular structure of lipids, such as cyclopropanation of cis double bonds, should strongly influence lipase-catalyzed hydrolysis of LLCNPs, a study showed that both the lipid ((1-(cis-9,10-methylene-octadecanoyl)-rac-glycerol), (1-(cis-cis-9,10–12,13-bis-methylene-octadecanoyl)-rac-glycerol), and (1-(cis-7,8-methylene-hexadecanoyl)-rac-glycerol)) molecular architecture and the geometry of the self-assembled nanoparticles govern enzymatic accessibility: the molecular structure affects engagement with the enzyme, while different mesophase geometries expose distinct regions of the lipid, altering headgroup accessibility. As a result, cyclopropanated lipids are generally hydrolyzed more slowly, and cubic phases are digested faster than hexagonal ones. These findings highlight that both molecular and nanostructural properties must be considered when designing mesosome-based systems for controlled delivery of drugs or nutrients [28].

On the whole, these studies discuss how enzymatic reactions may modify the lipid composition of pre-formed nanoparticles, producing amphiphilic species that alter the curvature stress within the membrane and trigger phase transitions. In both cases, the responsiveness of LLCNPs ultimately arises from the delicate balance between lipid molecular geometry, interfacial curvature, and the thermodynamics of self-assembly that governs the stability of inverse bicontinuous cubic mesophase.

Although only a few studies have specifically addressed enzyme-responsive cubosomes compared with other stimuli-responsive systems, the available results clearly demonstrate the potential of these nanostructured lipid carriers as adaptive soft materials capable of responding to biologically relevant enzymatic triggers. To validate their high pharmaceutical potential, future work in this area will likely focus on the rational design of LLCNP formulations incorporating enzyme-cleavable lipid motifs, the identification of disease-specific enzymatic triggers, and the integration of enzymatic responsiveness with controlled drug release or diagnostic functionality. Such developments could further expand the role of liquid crystalline nanoparticles as versatile platforms for stimuli-responsive nanomedicine.

H₂O₂-responsive

ROS represent another important trigger for stimuli-responsive nanocarriers, particularly in pathological environments such as inflammation and tumors, where oxidizing agents such as hydrogen peroxide (H₂O₂) are overexpressed. In this context, oxidation-responsive cubosome-like systems have recently been developed using functional block copolymers capable of undergoing chemical transformation upon exposure to oxidants. An example is provided by polymer cubosomes formed from highly asymmetric block copolymers composed of poly(ethylene oxide) and a ferrocene-containing block, such as PEO-b-PFcEMA (poly(2-(methacryloyloxy)ethyl ferrocene carboxylate)) [29]. These materials self-assemble into cubosome microparticles with a bicontinuous cubic Pn3m lattice. The presence of ferrocene units within the hydrophobic domain provides an intrinsic redox-responsive functionality. Upon exposure to oxidizing agents such as H₂O₂, the hydrophobic ferrocene groups are gradually oxidized into hydrophilic ferrocenium ions, converting the amphiphilic block copolymer into a more hydrophilic system. This chemical transformation induces swelling of the cubosome walls, enlargement of the internal pores, and progressive structural destabilization of the particles. Electron microscopy observations reveal that the oxidation process initially leads to pore expansion, followed by the formation of cracks and gradual disassembly of the cubosome architecture over time. The process is relatively slow because the oxidant must diffuse into the initially hydrophobic polymer matrix, but it ultimately results in particle degradation and release of encapsulated species. These findings demonstrate how the incorporation of redox-active groups into cubosome-forming polymers can yield H₂O₂-responsive nanostructures, providing a promising strategy for ROS-triggered delivery systems and for applications in oxidative biological environments.

Reversible addition-fragmentation chain transfer (RAFT)-mediated polymerization-induced self-assembly (PISA) provides a versatile platform for generating stimuli-responsive block copolymer nano- and macro-objects, particularly for oxidation-sensitive systems. Amphiphilic copolymers incorporating moieties such as thioether, selenide, or boronic esters exhibit responsiveness to ROS, notably H₂O₂, through oxidation-induced polarity changes that trigger morphological transitions or disassembly. During PISA, the growth of a hydrophobic block from a solvophilic precursor enables access to diverse morphologies, including V₂ and H₂ mesophases. Upon H₂O₂ exposure, oxidation increases hydrophilicity, altering the CPP and destabilizing the internal structure. This process can induce transitions from highly curved phases to disassembled states, with sensitivity tunable via polymer composition and architecture. These features are particularly relevant for

biomedical applications, where oxidative environments enable targeted payload release [30].

A related study reported polymer cubosomes prepared via RAFT-mediated PISA using poly(*N,N*-dimethylacrylamide) (PDMA) as the stabilizing block and a hydrophobic block containing boronic ester-functionalized monomers [31]. The boronic ester groups exhibit high sensitivity to H_2O_2 , undergoing oxidative cleavage to yield hydrophilic poly(acrylic acid). This transition from amphiphilic to hydrophilic character disrupts the V_2 nanostructure, leading to particle disassembly. Incorporation of aggregation-induced emission probes further enables real-time optical monitoring of this process, highlighting the potential of these systems as responsive nanocarriers.

These systems therefore represent H_2O_2 -responsive cubosomes, suitable for ROS-triggered release and sensing applications in oxidative biological environments.

Ionic-responsive

Ionic strength and specific ions can also act as triggers for structural transformations in LLCNPs. Changes in electrostatic interactions at the lipid–water interface can modify the CPP of amphiphilic molecules, thereby inducing phase transitions between lamellar, cubic, and other mesophases. Such ion-responsive behavior offers opportunities for developing smart delivery systems as well as simple sensing platforms based on structural changes in LLCNPs.

An illustrative example involves PT dispersions containing small amounts of ionic surfactants such as didodecyldimethylammonium bromide (DDAB) or dioctyl sulfosuccinate (AOT) [32]. PT typically forms cubosomes with a $\text{Pn}3\text{m}$ structure, but increasing surfactant content introduces electrostatic repulsion between charged headgroups, reducing negative curvature. This drives a structural transition from cubic phases ($\text{Pn}3\text{m} \rightarrow \text{Im}3\text{m}$) to lamellar vesicles, as confirmed by SAXS and cryo-TEM. These systems are also highly sensitive to ionic strength. Addition of salts screens electrostatic repulsions, decreasing the effective headgroup area and restoring negative curvature. As a result, lamellar structures reversibly transition back to cubic phases when exposed to environments characterized by high ionic strength. This effect is particularly pronounced with multivalent ions, which induce transitions at lower concentrations due to stronger electrostatic interactions. Overall, such lipid-surfactant mixtures demonstrate ion-responsive behavior, offering potential for stimuli-responsive drug delivery and sensing applications.

As a different strategy, protic ionic liquids (PILs) can be used to regulate the internal mesophases of MO-based

LLCNPs [33]. By tailoring cation and anion structures, PILs modulate lipid packing and interfacial curvature, enabling controlled transitions between H_2 , V_2 , and L_α or L_3 phases. These effects arise from ion partitioning at the lipid–solvent interface, altering effective headgroup area and membrane curvature. Importantly, PILs can significantly swell solvent channels and preserve the nanostructure upon freeze-drying, offering a versatile, non-competitive alternative to conventional additives for tuning drug loading and release in advanced nanocarriers.

Light-responsive

LLCNPs responsive to light can generally be divided into two major categories. The first includes systems that exploit light to trigger the isomerization of embedded photochromic molecules, which can induce a nanoparticle phase transition and thereby modulate controlled drug release. The second category comprises systems that use light to generate ROS through the irradiation of specific compounds known as PSs, ultimately causing cell death and thus enabling pharmaceutical applications such as photodynamic therapy.

The use of light as an external, non-invasive trigger to induce phase transitions in LLC systems has emerged as a powerful strategy for achieving on-demand drug release. This concept was first systematically explored by Boyd's group through the incorporation of photochromic molecules into bulk LLC phases. In an initial study, alkylated spiropyran derivatives such as spiropyran laurate were incorporated into PT-based matrices, demonstrating reversible switching between V_2 and H_2 phases upon UV irradiation, with a direct impact on drug release profiles [34]. Subsequent work thoroughly investigated spiropyran analogs with varying hydrophobic tail lengths, showing that molecular structure strongly influences phase transition temperatures, lipid packing disruption, and switching kinetics [35]. More recently, donor–acceptor Stenhouse adducts (DASAs) have been introduced as visible-light-responsive switches, enabling rapid and partially reversible phase transitions under green light irradiation, thus expanding the applicability of light-triggered LLC systems toward more biocompatible activation conditions [36].

These concepts were extended to LLCNPs through the development of a light-responsive system based on the incorporation of an alkylated DASA into a DLPC/lauric acid lipid matrix [37]. DASA undergoes reversible photoisomerization under green light (532 nm), switching from a hydrophobic, extended form to a more polar, compact structure. This change alters the CPP, inducing a structural reorganization of the lipid assembly. Time-resolved SAXS revealed a rapid and reversible transition from a L_α phase, associated with slow release (fluorescein isothiocyanate-dextran was used as

hydrophilic cargo), to an inverse bicontinuous cubic phase, characterized by higher diffusivity. This transition occurs within seconds upon irradiation and reverses in the dark. Functionally, this structural switch enables light-triggered release: about 8% of encapsulated cargo was released within 10 min of irradiation, increasing to ~20% after one hour, while negligible leakage occurred without light. Repeated on-off cycles demonstrated controlled, stepwise release. Unlike earlier systems where stimuli reduced release rates, this design enables a switch from a 'storage' to a 'release' state. The use of visible light further enhances its potential for minimally invasive, on-demand drug delivery.

Building on these findings, a complementary strategy was later explored using hexaarylbiimidazoles (HABIs) as photoresponsive actuators, offering distinct advantages in terms of photostability and structural control [38]. In this work, two derivatives, HABI1 and HABI2, were incorporated into PT- and DOPC-based LLC systems to investigate their ability to modulate lipid packing under visible light irradiation. HABIs exhibit rapid photoisomerization under green light, coupled with a significantly slower thermal reversion in the dark. Spectroscopic analysis confirmed fast and efficient switching, along with remarkable resistance to photofatigue. From a structural standpoint, time-resolved SAXS revealed that HABIs can induce pronounced and tunable phase transitions. In PT-based systems, irradiation promoted a V_2 -to- H_2 phase transition, with kinetics strongly dependent on HABI concentration. More importantly, by carefully tuning the lipid composition (notably DOPC/PT ratios), it was possible to engineer systems undergoing a lamellar to cubic transition, thus enabling a switch from slow to fast release regimes.

Chen et al. [39] employed cubosomes loaded with the photothermal agent indocyanine green for targeted melanoma therapy. Upon near-infrared irradiation, localized heating induced a phase change in the lipid membrane, increasing membrane permeability and promoting the release of the encapsulated chemotherapeutic payload. This approach enables spatiotemporal control over drug delivery, combining the intrinsic advantages of the ordered cubic nanostructure with light-responsiveness.

A further complementary approach to light-triggered control in LLCNPs exploits azobenzene-based photosurfactants, which introduce a distinct 'swell-squeeze' mechanism for cargo release [40]. In these systems, stable MO-based cubosome dispersions (~200 nm) exhibiting a V_2 phase were obtained over a broad compositional and temperature range. Notably, the cubic lattice parameter was highly sensitive to photosurfactants concentration: increasing loading induced lattice swelling, enabling fine tuning of the internal nanostructure, albeit at the expense of phase stability at higher concentrations. Upon UV irradiation, rapid

trans-cis isomerization of the azobenzene moiety resulted in a contraction of the cubic lattice. This effect is attributed to the increased effective tail volume and curvature of the amphiphile in the cis state, which compresses the internal structure. Importantly, this structural contraction was harnessed to trigger the release of hydrophobic guest molecules (e.g., Nile Red), with kinetics significantly faster than diffusion-controlled processes. This study introduces a mechanistically distinct paradigm, where light does not simply induce phase transitions but actively modulates lattice dimensions, providing an additional handle for designing responsive and tunable nanocarriers.

An alternative strategy involves photocleavable polymer cubosomes, where light induces structural disassembly rather than reversible phase transitions [19]. These systems are based on block copolymers (PEG45-b-PNBMAm) with high hydrophobic content (>90 wt%), forming well-defined internal nanostructures such as Im3m and Pn3m cubic phases and hexagonal phases. Upon light irradiation, cleavage of the photosensitive polymer backbone leads to irreversible breakdown of the nanostructure, enabling cargo release through carrier disintegration. This mechanism differs fundamentally from lipid-based systems, where release is typically governed by phase transitions or lattice modulation. Overall, this approach broadens the design of light-responsive nanocarriers, combining structural complexity with degradability. However, despite their potential, these systems currently lack *in vitro* or *in vivo* validation, limiting their demonstrated applicability in drug delivery. Indeed, it is worth noting that, although authors typically claim controlled drug delivery applications for these formulations, quite surprisingly, no *in vitro* and *in vivo* tests are often provided to support applications in the pharmaceutical field.

Another important category of LLC formulations, explored across several studies by the same authors, utilizes light to trigger ROS production for pharmaceutical applications. Photodynamic therapy (PDT) is a minimally invasive therapeutic approach that relies on the activation of a PS by light of a specific wavelength to generate ROS, which selectively damage pathological cells while sparing healthy tissue. This modality has been increasingly explored for cancer treatment due to its high spatial and temporal control, reduced systemic toxicity, and the possibility of combining it with other therapies. The efficacy of PDT is strongly influenced by the solubility, stability, and intracellular delivery of PSs, which has prompted the development of innovative nanocarrier systems capable of protecting and efficiently transporting hydrophobic PSs into target cells. Among these, lipid-based LLCNPs have emerged as promising platforms for encapsulation of PSs, offering tunable nanostructures, high encapsulation efficiency, and enhanced bioavailability.

Initial work demonstrated the feasibility of using LLCNPs as carriers for photodynamic therapy [41]. In this pioneering work, hydrophobic PSs were successfully loaded into MO-based cubosomes for the treatment of human skin melanoma. Cubosomes showed efficient encapsulation of the PSs (chlorin e6 or meso-Tetraphenylporphine-Mn(III) chloride), while bio-imaging studies demonstrated that the encapsulated cargo localized within the cytoplasm of MeWo and Me45 melanoma cells, confirming the strong potential of the developed cubosomes for skin cancer diagnostics. Moreover, the encapsulated chlorin e6 exhibited excellent biocompatibility and high photodynamic activity, achieving over 90% reduction in cell viability for both MeWo and Me45 cells after irradiation. Notably, this photodynamic effect was even more efficient than that obtained with the free PS administered at a concentration ten times higher. This foundational work provided the proof-of-concept that cubosomes can be used as light-responsive drug delivery systems for PDT applications, setting the stage for subsequent studies aiming at combining green extraction of natural PSs with advanced nanocarrier technology.

The second study extended these concepts by investigating multifunctional cubosomes engineered for combined photodynamic and chemotherapeutic cancer treatment [42]. A diketopyrrolopyrrole–porphyrin PS was conjugated to the block copolymer PF108, which stabilized the nanoparticles, while the chemotherapeutic agent docetaxel (DTX) was encapsulated within the lipid bilayer. Physicochemical characterization by SAXS, DLS, and cryo-TEM confirmed the presence of stable cubosomes (~ 159 nm) with a V_2 structure (Pn3m and Im3m phases). Confocal imaging revealed localization of the fluorescent cubosomes in lysosomes and mitochondria, highlighting effective intracellular delivery. Cytotoxicity studies on HeLa cells showed that cubosomes without DTX were non-toxic, whereas DTX-loaded cubosomes reduced cell viability significantly. Upon irradiation, the PS induced additional phototoxicity, demonstrating a slight but meaningful synergistic effect with DTX. These findings indicate that surface-conjugated PSs in cubosomes, combined with encapsulated chemotherapy, can enhance therapeutic efficacy while maintaining structural stability, offering a promising strategy for theranostic nanomedicine applications.

A different approach explored hybrid theranostic cubosomes engineered for Near-Infrared (NIR)-triggered PDT and chemotherapy [43]. The lipid-based nanoparticles were co-loaded with daunorubicin (DNR) as a chemotherapeutic and PS, and with up-converting $\text{NaYF}_4:\text{Er}^{3+}, \text{Yb}^{3+}$ nanoparticles (UCNPs) serving as energy harvesters and imaging agents. Surface functionalization was achieved through a layer-by-layer assembly of chitosan, single-strand DNA, and folic

acid–chitosan conjugate, providing stability, biocompatibility, and cancer-targeting capability. Physicochemical characterization confirmed the preservation of the bicontinuous cubic Im3m nanostructure, with sizes between 154 and 169 nm and tunable ζ -potential values depending on the outer layer. Cellular studies in SKOV-3 (ovarian) and MeWo (melanoma) cells demonstrated enhanced uptake of folic acid-functionalized cubosomes, particularly in SKOV-3 cells, leading to efficient intracellular delivery. Upon NIR irradiation, Förster resonance energy transfer (FRET) between UCNPs and DNR triggered singlet oxygen generation, reducing mitochondrial activity and causing cytoskeleton disruption.

More recently, a study focused on the development of Ru(II) polypyridyl complexes (RPCs) as PSs for PDT [44]. By introducing two π -expansive dppn ligands simultaneously coordinated to the Ru(II) center, the authors obtained the two complexes Ru1 and Ru2, which exhibit enhanced singlet oxygen sensitization capabilities, resulting in potent light-triggered cytotoxicity against epidermoid carcinoma cells. To improve biopharmaceutical properties, both complexes were encapsulated in MO-based cubosomes, with Ru2-loaded cubosomes showing superior encapsulation efficiency and colloidal stability. In vitro experiments confirmed effective intracellular ROS generation upon light irradiation, supporting the phototoxic mechanism. This work represents the first example of RPCs incorporated into cubosome nanostructures, highlighting the potential of bis-dppn Ru(II) complexes for the rational design of nanoformulated PSs with enhanced therapeutic applicability in PDT.

In a related study, an MO-based cubosome formulation loaded with a different, newly synthesized ruthenium-based PS (PS–Ru) was tested by the same authors [45]. Photophysical characterization revealed a strong singlet oxygen generation capacity ($\phi\Delta = 0.54 \pm 0.06$). Encapsulation of PS–Ru in cubosomes stabilized by a biodegradable analog of PF127 produced nanoparticles of ~ 148 nm with high loading efficiency ($\sim 90\%$) and preserved Pn3m structure, as confirmed by cryo-TEM and SAXS. Particle size and surface charge remained stable across temperature variations and over time, indicating excellent colloidal stability. Biological studies in Calu3 lung adenocarcinoma cells demonstrated potent phototoxicity of PS–RuCub under irradiation, with IC_{50} values in the nanomolar range and minimal dark toxicity, while empty cubosomes were non-toxic.

Most recently, a study further demonstrated the translational potential of cubosome-based PDT systems using a natural PS derived from marine algae [46]. The microwave-assisted ethanol extraction of *Ulva rigida* yielded a hydrophobic extract rich in chlorophyll *a* ($0.98 \pm 0.09\%$ of dry weight), chlorophyll

b ($0.093 \pm 0.008\%$), and lutein ($0.92 \pm 0.01\%$). This extract was successfully encapsulated in MO-based bile salt-sorbitan monooleate-stabilized cubosomes (TS-CUB). SAXS and cryo-TEM analysis confirmed that TS-CUB exhibited coexisting Pn3m and L_3 (sponge) phases. ROS assays demonstrated enhanced photoactivity for TS-CUB, producing up to 15% ROS reduction at $8.8 \mu\text{g/mL}$, surpassing the free extract. In BxPC-3 pancreatic cancer cells, TS-CUB + extract displayed superior cellular uptake, minimal dark cytotoxicity, and strong photodynamic cytoskeleton disruption post-irradiation, highlighting its potent PDT efficacy.

According to the papers presented in this paragraph, light-responsive cubosomes offer versatile strategies for on-demand drug release and photodynamic therapy.

Magneto-responsive

Magnetically responsive cubosomes (magneto-cubosomes) represent an attractive class of remotely controlled drug delivery systems in which structural changes and cargo release can be triggered by an external magnetic field [47]. This strategy typically relies on the incorporation of superparamagnetic iron oxide nanoparticles (SPIONs) within the lipid matrix or bilayer of the cubic phase. Upon exposure to an alternating magnetic field (AMF), these magnetic nanoinclusions generate localized heating through Néel and Brownian relaxation processes, which can induce changes in membrane permeability or trigger phase transitions within the liquid crystalline structure. As a result, magneto-cubosomes enable non-invasive, spatially and temporally controlled activation of drug release.

An important milestone in the development of such magnetically responsive liquid crystalline nanoparticles was the first demonstration of magneto-cubosomes [48]. In this work, cubosomes based on MO were engineered to include hydrophobic Fe_3O_4 nanoparticles coated with oleic acid and oleylamine embedded in the lipid bilayer of the bicontinuous cubic phase. SAXS confirmed that the incorporation of the magnetic nanoparticles preserved the internal Pn3m cubic symmetry, although a slight contraction of the lattice parameter was observed. The hybrid bulk cubic phase was subsequently dispersed with PF127 to produce stable magneto-cubosomes with an average hydrodynamic diameter of about 200 nm, comparable to conventional cubosomes. This study represents the earliest demonstrations that the lipid bilayer of cubic phases can accommodate magnetic nanoinclusions (approximately 6 nm in size) while maintaining the V_2 architecture. The presence of SPIONs imparted magnetic responsiveness, enabling remote activation through a low-frequency AMF. Under magnetic stimulation, localized heating generated by relaxation processes of the nanoparticles was proposed to induce transient perturbations in the lipid bilayer,

promoting the release of hydrophilic molecules confined within the aqueous channels. Using fluorescence correlation spectroscopy, the authors showed that application of the magnetic field significantly accelerated the release of a model hydrophilic probe compared with spontaneous diffusion from the cubic matrix. Importantly, this work provided the first clear evidence of magnetically triggered release from cubosomes, establishing magneto-cubosomes as a new class of hybrid lipid nanocarriers capable of externally controlled, spatially and temporally regulated drug release.

In a subsequent study from the same group [49], the structural effects of incorporating hydrophobic Fe_3O_4 nanoparticles into MO liquid-crystalline assemblies were investigated in greater detail. SAXS revealed that increasing SPIONs content progressively altered the polymorphic behavior of the lipid mesophase. While the native MO/water system forms a Pn3m bicontinuous cubic phase, the presence of SPIONs promoted a V_2 to H_2 transition, indicating that the nanoparticles modify both the elastic curvature and packing frustration of the lipid bilayer. Importantly, the transition temperature decreased significantly with increasing SPIONs concentration, shifting from about 90°C in the binary MO/water system to near physiological temperatures. When these hybrid phases were dispersed as cubosomes, similar thermotropic behavior was observed. Moreover, in situ SAXS experiments under an AMF demonstrated that SPIONs-induced local heating could trigger structural changes in the cubic phase, providing direct evidence that magnetically responsive lipid mesophases can undergo phase transitions associated with the controlled release of encapsulated molecules.

The heat produced by MO/SPIONs-based magneto-cubosomes under an AMF induces a V_2 -to- H_2 phase transition in the lipid matrix. This reorganization triggers water expulsion, increases membrane permeability, and drives linear clustering of SPIONs, enhancing their magnetic response for hyperthermia applications. Consequently, these magneto-cubosomes enable on-demand release of hydrophilic drugs. Moreover, they are efficiently internalized by HT29 cancer cells, and exhibit minimal cytotoxicity, demonstrating their potential as multifunctional, stimuli-responsive nanocarriers for targeted therapy [50].

In another study, magnetic nanoparticles with different surface properties were incorporated into MO-based mesophases [51]. Hydrophilic nickel-zinc ferrite nanoparticles coated with citric acid localized within the aqueous channels, while hydrophobic iron oxide nanoparticles coated with oleic acid partitioned into the lipid bilayer. SAXS analysis showed that the Pn3m cubic phase was preserved up to 2% w/w nanoparticle loading at physiological temperature. Interestingly, the authors reported that hydrophilic NPs lowered the V_2 -to- H_2

transition temperature to 40 °C. While the authors attributed this behavior to water channel swelling, such a mechanism typically stabilizes phases with lower inverse curvature; thus, the exact molecular driving force favoring the H_2 phase in this system remains to be fully clarified. In contrast, hydrophobic nanoparticles had minimal impact. Cubosomes stabilized with PF127 (≈ 110 – 150 nm) were obtained. Drug release studies with doxorubicin (DOX) showed that hydrophilic nanoparticles slowed release via electrostatic interactions, while hydrophobic ones had little effect.

The same group also investigated methotrexate (MTX) delivery using hybrid systems prepared by incorporating hydrophobic iron oxide nanoparticles into the lipid domain of an MO-based bicontinuous cubic phase [52]. These nanoparticles (≈ 7 nm) localized mainly within the lipid bilayer without obstructing the aqueous channels, allowing MTX diffusion. The cubic phase was then dispersed into cubosomes and magneto-cubosomes, which retained the internal cubic structure. Application of a low-frequency AMF significantly accelerated MTX release. This effect was attributed to mechanical vibrations of the embedded magnetic nanoparticles disrupting the internal structure of magneto-cubosomes, rather than to magnetothermal heating.

Magnetically responsive lipid–liquid-crystalline systems incorporating SPIONs have been also explored by Boyd's group. In a first study, both bulk and dispersed PT-based liquid crystalline systems doped with SPIONs were investigated to determine how nanoparticle size, surface chemistry, and concentration influence magnetically induced phase transitions and drug release. The PT/water system forms a $Pn3m$ phase at room temperature, which can transform into a H_2 phase upon heating. When SPIONs were incorporated, the oscillatory motion of the nanoparticles under AMF generated local heating via Brownian and Néel relaxation mechanisms, triggering the V_2 -to- H_2 transition. The magnetically induced transition could occur within minutes and was particularly efficient in bulk lipid matrices, where even low nanoparticle concentrations (0.01–0.1 wt%) enabled rapid and reversible phase switching. Importantly, this structural transition directly influenced drug release kinetics. *In vitro* experiments demonstrated that the AMF-induced V_2 -to- H_2 transformation modulated diffusion-controlled release from the cubic matrix, producing a magnetically controllable drug–release profile [53].

A related but conceptually different strategy was presented in a second study, where the lipid composition was engineered to reverse the direction of the release response. In this work, PT was mixed with the PEGylated phospholipid DSPE-PEG2000 to modify the CPP of the lipid system and produce a L_α phase at room temperature rather than a cubic phase. At an optimized molar ratio (PD5, PT:DSPE-PEG2000 = 95:5), the

system underwent a thermally driven transition from L_α to $Im3m + H_2$ phases mixture at approximately 35 °C with a complete transition to the $Im3m$ phase at above 65 °C. Incorporation of SPIONs enabled this phase transformation to be triggered remotely by AMF, again through magnetically generated local heating. Structural studies using SAXS showed that the oscillation of the nanoparticles raised the internal temperature of the lipid matrix to about 40 °C, initiating the lamellar-to-cubic transition within several minutes. In contrast to the previously described system [53], where magnetic activation slowed release by converting the cubic into the more diffusion-restrictive hexagonal phase, the PD5 formulation produced the opposite behavior: negligible release in the 'off' state (lamellar liposomes) and accelerated release in the 'on' state following AMF-induced formation of cubic nanostructures. Drug-release experiments confirmed this concept, demonstrating magnetically triggered release of the model hydrophilic compound sulfo-Cy5 from PEGylated liposomes containing SPIONs [54].

Taken together, the two studies highlight complementary design strategies for magnetically responsive lipid nanocarriers. While the first investigation [53] demonstrates how embedded SPIONs can modulate the internal mesophase of pre-formed V_2 systems to control diffusion pathways, the second [54] illustrates how tuning lipid composition allows the magnetic trigger to induce a L_α -to- V_2 transition that activates drug release. Both approaches rely on magnetothermal heating generated by nanoparticle dynamics under AMF, but they achieve different release behaviors, emphasizing the importance of coupling nanoparticle properties with lipid packing geometry to engineer magnetically controlled delivery systems.

In another recent study, MO-based magneto-cubosomes incorporating SPIONs and stabilized with PF127 were used to encapsulate the antitumor compound helenalin [55]. Here, the magnetic responsiveness was introduced to enable external magnetic guidance and potential magnetically assisted targeting. The resulting nanoparticles (≈ 180 – 190 nm) retained a well-defined $Im3m$ bicontinuous cubic internal structure, as confirmed by SAXS and cryo-TEM, with encapsulation efficiencies of around 70%. Cytotoxicity assays showed that empty cubosomes were essentially non-toxic toward rhabdomyosarcoma (RMS) cells, maintaining cell viability above 95% at concentrations up to 20 $\mu\text{g}/\text{mL}$, confirming the biocompatibility of the lipid carrier. Helenalin-loaded cubosomes significantly enhanced the antiproliferative activity of the drug compared with the free molecule. For example, while 1.5 μM free helenalin resulted in RMS cell viability of about 92%, the same dose encapsulated in cubosomes reduced viability to $\sim 73\%$. To further increase selectivity, the magnetic cubosomes were functionalized with hyaluronic acid targeting the CD44 receptor and with anti-CD221

antibodies, both overexpressed in RMS cells. These magnetically responsive, dual-targeted cubosomes maintained the cubic structure while exhibiting enhanced cellular uptake. The triple-functionalized magneto-cubosomes reduced RMS cell viability to about 50% at 1.5 μM helenalin and to $\sim 27\%$ at 2.5 μM , markedly lower than that observed for the free drug, while toxicity toward fibroblasts remained comparatively limited. While the incorporation of SPIONs demonstrates the structural feasibility of magnetically responsive cubosomes, the magnetic functionality remains largely conceptual in this study, as magnetic guidance or magnetothermal activation were not experimentally investigated. Notably, the biological evaluation was here performed using three-dimensional tumor spheroids, providing a more physiologically relevant assessment of the therapeutic potential of these multifunctional nanocarriers.

pH-responsive

pH-responsive LLCNPs were first investigated about a decade ago. However, research activity in this area has markedly increased over the past five years, making them the most extensively studied class of stimuli-responsive LLCNPs. pH-responsiveness in cubosomes and hexosomes can be achieved through several complementary design strategies that exploit changes in molecular charge as a function of pH [10]. Indeed, variations in pH can alter intermolecular interactions within the lipid assembly, inducing phase transitions and enabling controlled payload release. Approaches to impart pH sensitivity include the use of ionizable lipids, pH-responsive block copolymers, anionic additives, and pH-sensitive drugs or prodrugs. These components can modulate the curvature and stability of the nanostructure, thereby tuning release behavior.

It is important to note that even conventional MO-based cubosomes can exhibit intrinsic pH-responsive behavior without the need for additional functional components [56]. Specifically, DOX-loaded cubosomes showed accelerated drug release under mildly acidic conditions ($\text{pH} \approx 5.4$), mimicking the tumor microenvironment, while remaining relatively stable at neutral pH. This passive pH sensitivity translated into enhanced anticancer efficacy, as lower drug doses were required to inhibit glioblastoma cell proliferation compared with free DOX. Similarly, incorporation of wedge-shaped unsaturated FAs in MO-based systems enables the formation of inverse micellar cubic (Fd3m symmetry) cubosomes, representing a less explored but promising architecture for sustained drug release. In these systems, the phase behavior is strongly influenced by pH, as well as by temperature and fatty acid composition, which collectively govern structural stability and phase transitions [57].

pH-responsive cubosomes can be engineered through specific drug–lipid interactions, as shown for MO-based systems loaded with nicergoline [58]. In this case, the ionization state of the drug modulates the internal cubic structure: at high pH, the neutral form favors transitions from cubosomes to inverse micellar or hexosome-like structures, whereas at low pH, protonation promotes a shift toward vesicular structures. At intermediate pH, changes in the lattice parameter of the V_2 phase are observed. These reversible transitions highlight that drug properties may add complexity to their rational design for controlled release applications.

Another study demonstrates that pH-responsiveness in cubosome/hexosome systems can arise directly from lipid–peptide (LL-37) interactions, without the need for synthetic responsive additives [59]. In oleic acid/LL-37-based formulations, the gradual deprotonation of the lipid with increasing pH modulates electrostatic repulsions and alters the CPP, driving a complex sequence of structural transitions, including micellar cubosomes, hexosomes, vesicles, and micelles. Notably, ordered nanostructures such as cubosomes are only stable within a relatively narrow pH and compositional window, while higher peptide loading promotes disorder and phase coexistence. Another work by the same group further demonstrates how pH-responsiveness in cubosomes can be achieved by incorporating an ionizable drug directly into the lipid matrix [60].

In a formulation composed of MO and the anticancer drug 2-hydroxyoleic acid, the protonation state of the fatty acid governs curvature and phase behavior: at low pH ($\text{pH} < 4$), the neutral form of the anticancer drug partitions into the hydrophobic domain of the lipid (MO) matrix, promoting more negatively curved structures (hexosomes), while at intermediate pH (~ 4) stable cubosomes are formed. Upon further increases in pH ($\text{pH} > 4.5$), deprotonation induces electrostatic repulsion at the interface leads to swelling of the cubic phase and eventual transition to vesicles.

Cubosomes co-loaded with DOX and *Brucea javanica* oil demonstrated promising pH-responsive behavior for anticancer therapy. Structural characterization revealed a reversible phase transition from inverted hexagonal phases at physiological pH (7.4) to cubic networks under mildly acidic conditions ($\text{pH} < 6.8$), and further to emulsified microemulsions at endosomal/lysosomal pH (≈ 5.3). These transformations correlate with controlled, sustained drug release and enhanced interaction with lipid membranes in acidic microenvironments, mimicking tumor tissues. In vitro studies on MCF-7 cells showed that dual-drug-loaded cubosomes induced stronger apoptotic effects and potentially overcame DOX resistance compared with free drug suspensions [61].

In the context of antifungal therapy against *Cryptococcus neoformans*, ionizable lipid formulations enable reversible switching from neutral hexosomes at physiological pH to cationic cubosomes under acidic conditions. This transition enhances electrostatic interactions with the negatively charged fungal cell wall, significantly improving drug efficacy. Such responsiveness leads to reduced minimum inhibitory concentration values and pronounced cellular damage, highlighting the potential of stimuli-responsive LLCNPs to overcome drug resistance through environment-triggered targeting and release [62].

The incorporation of the pH-sensitive polymer (the pseudopeptide PP50) into MO-based cubosomes provides an effective strategy to impart them environmental responsiveness. While the Im3m structure is preserved and swollen at physiological pH, polymer–lipid interactions under acidic conditions induce significant structural disruption, with coexistence of disordered, cubic, and lamellar domains. This transition is attributed to polymer penetration within water channels and modulation of membrane curvature elasticity [63]. This behavior highlights a key design principle: introducing responsive stabilizers enables structural destabilization specifically in acidic environments, providing a promising route for controlled drug release in pathological conditions such as tumors or endosomes.

In a series of papers Demetzos' group investigated the pH-responsive behavior imparted to cubosomes by substituting PF127 used for the nanoparticle stabilization in water with other polymers. Initial studies employing poly(2-(dimethylamino)ethyl methacrylate)-*b*-poly(lauryl methacrylate) (PDMAEMA-*b*-PLMA) demonstrated that this amphiphilic copolymer can effectively stabilize MO-based LLCNPs, provided that appropriate conditions, such as full protonation or the presence of co-stabilizers, are met [64]. These systems exhibit a pronounced pH-dependent modulation of surface charge, driven by the ionizable amino groups, which primarily affects interfacial properties and morphology rather than particle size. However, they also reveal intrinsic limitations, as increased polymer content or environmental perturbations (e.g., ionic strength and proteins) can compromise structural integrity.

A direct comparison with conventional stabilizers further highlighted that PDMAEMA-*b*-PLMA can match the performance of PF127 in MO systems, while introducing additional functionality such as enhanced electrostatic interactions with biological interfaces [65]. Nonetheless, this advantage is strongly lipid-dependent, with PT-based systems displaying reduced stability and a higher propensity for aggregation, underscoring the delicate balance between responsiveness and robustness.

Building on these findings, more advanced multifunctional copolymers incorporating targeting moieties, such as poly(2-(dimethylamino)ethyl methacrylate)-*b*-poly(lauryl methacrylate) block copolymer carrying triphenyl-phosphine cations (TPP-QPDMAEMA-*b*-PLMA), were developed to integrate pH-responsiveness with subcellular targeting capabilities [66]. These systems exhibit high positive ζ -potentials and preferential lysosomal accumulation, but their performance depends critically on the interplay between different stabilizers, which governs protein interactions, morphology, and overall stability [67].

A similar approach was used in two studies by Drummond et al. that introduced a new generation of amphiphilic block copolymers containing a hydrophilic poly(ethylene glycol) block, a hydrophobic block and one or two responsive blocks, i.e., poly(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl acrylate)) and/or poly(2-(dimethylamino)ethyl methacrylate), capable of simultaneously stabilizing LLCNPs and imparting multi-stimuli responsiveness. Unlike conventional approaches relying on separate stabilizers and ionizable additives, these polymers integrate both functions, enabling control over the internal nanostructure in response to pH, temperature, and H₂O₂ [68]. Additionally, MO-based cubosomes were formulated incorporating pH-responsive PDMAEMA and H₂O₂-responsive poly(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl acrylate) (PTBA) blocks, enabling high paclitaxel encapsulation (~60%) and triggering drug release under tumor-mimicking conditions (pH 5.5 and oxidative environments) [69]. Compared with physiological pH, release rates were significantly enhanced, reflecting polymer degradation or solubility changes. Importantly, these systems also exhibited improved cellular uptake and higher cytotoxicity than free drug or non-responsive formulations. Overall, these works highlight how functional block copolymers can go beyond stabilization, enabling synergistic control of responsiveness, drug release, and biological interactions in advanced cubosome-based nanocarriers.

A complementary strategy to engineer pH-responsive cubosomes relies on the incorporation of biopolymer-based polyelectrolyte shells [17,70–72]. These core–shell LLCNPs, composed of a bicontinuous cubic lipid core and a chitosan-based polyelectrolyte coating (N-arginine-modified chitosan/alginate, CHarg), represent advanced adaptive soft matter systems capable of dynamically responding to complex biological environments such as the gastrointestinal tract. The presence of ionizable groups within the shell confers pH-dependent charge, enabling modulation of interfacial properties, mucoadhesion, and nanoparticle swelling. MO-based cubosomes functionalized with CHarg maintain their internal cubic organization while

exhibiting enhanced mucosal interaction and retention, highlighting the role of the shell in stabilizing and functionally tuning the nanostructure [70]. Under gastric conditions, the polyelectrolyte shell provides a protective barrier against enzymatic degradation and harsh acidity, preserving the internal mesophase and limiting premature cargo release. In contrast, intestinal environments trigger pronounced structural transformations, including V_2 -to- H_2 transitions and eventual disassembly, primarily driven by bile salts and enzymatic activity [72]. Importantly, the interplay between environmental pH, digestive biomolecules, and cargo concentration governs the structural evolution and release pathways of these systems, emphasizing their highly adaptive behavior.

Beyond pH responsiveness, specific biomolecular interactions further modulate cubosome structure. For instance, binding of serum proteins such as albumin can induce significant internal rearrangements, including transitions from $Im3m$ to $Pn3m$ symmetry and contraction of aqueous channels, promoting water expulsion and facilitating drug release [71]. This highlights the importance of considering protein corona effects in the design of responsive LLCNPs. On the other hand, encapsulation of bioactive molecules, such as the polysaccharide acemannan, introduces an additional level of structural control. The payload itself can influence mesophase behavior, inducing partial transitions toward H_2 phases at physiological pH, reducing lattice spacing and enabling triggered expulsion of the aqueous cargo [17]. Furthermore, these hybrid systems exhibit strong, curvature-dependent interactions with lipid membranes, suggesting the potential for membrane fusion, modulation, and enhanced cellular uptake. As the colon represents a key target for oral drug delivery, yet gastrointestinal degradation severely limits therapeutic efficacy, an article investigated a pH-responsive delivery platform developed using PT-based cubosomes embedded in microbial nanocellulose capsules and coated with alginate [73]. This system enables protection during gastrointestinal transit and controlled, site-specific release. The bicontinuous cubic structure allows simultaneous encapsulation of hydrophilic and hydrophobic compounds, supporting multidrug delivery. The integration of cubosomes with biofabricated cellulose capsules provides a versatile, non-toxic platform for colon-targeted, pH-triggered oral administration of bioactives.

The design of pH-responsive LLCNPs has been effectively advanced through the incorporation of ionizable aminolipids into MO-based systems [74,75]. Nine aminolipids were synthesized and shown to modulate the internal mesophase of nanoparticles in a pH-dependent manner, inducing a transition from H_2 to V_2 structures within the pathophysiologically relevant pH range (≈ 3.5 – 6.5). This transition is driven by

protonation of the aminolipid headgroups under acidic conditions, which increases their effective size and reduces interfacial curvature, ultimately promoting structural reorganization [74]. The practical application of such systems was demonstrated by developing aminolipid-doped nanoparticles capable of encapsulating the poorly soluble chemotherapeutic SN-38. These nanocarriers exhibited high drug loading and a marked pH-triggered release, with slow release at physiological pH and accelerated release under acidic tumor-like conditions. Notably, the H_2 -to- V_2 transition could be finely tuned by aminolipid structure and composition, even in the presence of the drug [75].

The design of micellar cubosomes with $Fd3m$ symmetry using MO nanoparticles doped with unsaturated fatty acetates (FACes) and FAs was also explored. High-throughput formulation and synchrotron SAXS demonstrated that FACes alone promoted stable $Fd3m$ cubosomes across a broad pH range (2.6–8) and temperatures up to 45 °C. Co-doping FACes with ionizable FAs enabled fine-tuning of the $Fd3m$ -to- H_2 phase transition pH between 5 and 7, allowing control over drug release, as H_2 phases generally release cargo faster than $Fd3m$ phases. These results suggest that manipulating the balance between non-ionizable and ionizable lipids provides precise control over nanostructure stability, pH-responsiveness, and release kinetics [76].

A study investigated pH-responsive LLCNPs formulated with ionizable lipids used in mRNA vaccines (ALC-0315 and SM-102) combined with PT [77]. By constructing partial phase diagrams as a function of lipid composition and pH, the authors demonstrated a clear pH-driven mesophase evolution from disordered structures to inverse micellar, hexagonal, and cubic phases upon acidification (pH 7 $\rightarrow$ 4), mimicking endosomal maturation. These transitions reflect a progressive decrease in interfacial curvature driven by lipid protonation, which also modulates the apparent pKa of the system. Importantly, the choice of stabilizer (PF127, PF108, or Tween 80) further influences phase behavior.

The same group developed pH-responsive stabilizers to tailor the structural and functional behavior of LLCNPs. Amphiphilic block copolymers incorporating polyethylene glycol, hydrophobic alkyl chains, and ionizable groups such as 2-(dimethylamino)ethyl methacrylate or diethylaminoethyl methacrylate enabled fine control over membrane curvature and mesophase organization. These systems stabilize MO- or PT-based nanoparticles while introducing pH-dependent transitions between V_2 , H_2 , and L_α phases. Such structural modulation directly impacts drug release kinetics, with phase transitions enabling tunable release profiles. Notably, responsiveness to mildly acidic environments, such as the tumor microenvironments, enhances targeting while

minimizing off-target effects [78]. Importantly, Drummond's group systematically compared clinically approved COVID-19 vaccine lipid NPs and design MO-based formulations to improve performance [79]. Incorporating MO as a structural helper lipid induces pH-dependent mesophase transitions, promoting H₂ structures that enhance mRNA release and transfection. MO-based NPs outperform Moderna lipid NPs *in vitro* across multiple cell types, including lung macrophages, epithelial cells, and cancer cells, achieving >90% transfection efficiency with optimized 30MO formulations. SAXS and cryo-TEM analyses reveal that MO substitution generates a uniform internal non-lamellar structure, resistant to protein corona effects, facilitating endosomal escape. *In vivo*, intranasal administration of 30MO-LNPs increases lung deposition, while intravenous delivery enables spleen-specific mRNA expression, highlighting the influence of lipid composition and administration route on organ targeting. This study establishes clear structure–property–function relationships, demonstrating that MO-induced mesophase transitions and protein corona resilience enhance intracellular delivery.

Considering the growing challenge of antibiotic-resistant bacteria, which has driven the development of alternative antimicrobial materials, an article reports a biocompatible, pH-responsive system combining bacteria-derived rhamnolipids (RLs) and food-grade MO. RL is solubilized within the V₂ MO core, forming stable supramolecular nanostructures that undergo reversible, pH-triggered transformations. The RL/MO vesicles selectively kill *Staphylococcus aureus* and methicillin-resistant *S. aureus* at acidic pH, inhibit biofilm formation, and remain non-toxic to human cells at neutral pH, offering a promising platform for targeted antimicrobial applications [80].

Gröeshel and his collaborators investigated poly(4-vinylpyridine)-based systems that introduce pH-responsive pyridinic moieties within the cubosome walls, enabling both structural adaptability and active molecular interactions [81]. These porous polymer cubosomes form through controlled self-assembly and exhibit interconnected channel networks that facilitate mass transport and high loading capacity. Importantly, their responsiveness to pH allows reversible modulation of stability, enabling triggered disassembly and release of encapsulated species under acidic conditions. Additionally, the presence of coordination sites permits incorporation of metal ions or nanoparticles, extending functionality toward catalysis and sensing.

Another recent study highlighted the importance of correlating mesophase transitions with lipid membrane physicochemical properties in pH-responsive lipid nanoparticles. In this work, nanoparticles based on 2-hydroxyoleic acid and MO were shown to undergo a pH-

triggered transition from lamellar vesicles to V₂ phases (Im3m/Pn3m), as confirmed by SAXS. Importantly, this structural transformation was accompanied by significant changes in membrane properties, including increased lipid packing, chain torsion, and variations in interfacial polarity and membrane fluidity, as revealed by fluorescence probes and Raman spectroscopy [82]. pH-responsive, stabilizer-free liquid crystalline nanoparticles based on positional isomers of diolein combined with the anticancer agent 2-hydroxyoleic acid offer tunable internal architectures, ranging from lamellar to inverse hexagonal and micellar cubic Fd3m phases. Their structural transformations depend on lipid composition, pH, molecular shape, ionization state, and interfacial interactions, as characterized by SAXS, cryo-TEM, and nanoparticle tracking [18]. These findings highlight the potential of such biocompatible nanoplatforms for controlled and targeted delivery of 2-hydroxyoleic acid and other therapeutic agents.

Inefficient intracellular delivery limits nanomedicine development for brain disorders. An article reports a pH-responsive lipid nanoparticle platform (PUFA-LNPs) based on MO, ionizable ω -3 FAs, co-encapsulating ginkgolide B and quercetin and functionalized with the cell-penetrating peptide Pituitary adenylate cyclase-activating polypeptide for targeted neuronal delivery. The hexosomal LC-LNPs undergo rapid, pH-triggered structural changes, enhancing intracellular release and synergistic neuroprotection. *In vitro* and *in vivo* studies demonstrate mitochondrial support, antioxidant activation, and safety, highlighting the potential of PUFA-LNPs as a dynamic, nose-to-brain nanomedicine strategy for neurodegenerative and oxidative stress-related therapies [83].

In conclusion, pH-responsive LLCNPs represent a highly versatile and rapidly evolving platform for controlled drug delivery. A wide range of strategies — spanning ionizable lipids, responsive polymers, and biopolymer coatings — enable precise tuning of nanostructure, surface properties, and release behavior in response to physiological cues.

Thermo-responsive

Thermo-responsive nanoparticles are designed to undergo structural or physicochemical changes in response to temperature variations. In liquid crystalline nanoparticles, thermal transitions can modify the internal architecture or the conformation of thermosensitive polymer components, leading to order–disorder transitions or channel size variations. These temperature-induced structural changes can be exploited to regulate nanoparticle stability and enable temperature-triggered release of encapsulated cargo.

A temperature-responsive system analogous to cubosome-like nanostructures by using amphiphilic block copolymers was reported [84]. The

semicrystalline copolymer poly(ethylene oxide)-block-poly(octadecyl methacrylate) (PEO₃₉-b-PODMA₁₇) self-assembles in water into nanoparticles with diameters of a few hundred nanometers, prepared via solvent exchange and dialysis. Cryo-TEM and cryo-electron tomography revealed an ordered bicontinuous internal morphology at low temperature, consisting of a sponge-like network of aqueous and polymer-rich channels (~13 nm), closely resembling lipid cubosomes. Differential scanning calorimetry identified a thermal transition at ~22 °C, corresponding to melting of the PODMA side chains. Above this temperature, the nanoparticles undergo an order–disorder transition, with loss of internal organization and a morphological shift toward flattened structures. Fluorescence studies using pyrene confirmed changes in polarity and microviscosity across the transition. These findings indicate that chain crystallinity stabilizes the ordered structure, while melting induces disorder. Such polymeric nanoparticles represent cubosome analogs with temperature-responsive behavior, offering potential for controlled release and templating applications.

Another strategy to introduce thermal responsiveness in liquid crystalline nanoparticles involves thermoresponsive polymer stabilizers [85]. In this approach, MO-based cubosomes are stabilized with poly(N,N-dimethylacrylamide)-block-poly(N-isopropylacrylamide) (PDMA-b-PNIPAM), replacing conventional PF127. Structural and colloidal properties, investigated by DLS, SAXS, QCM-D (quartz crystal microbalance with dissipation monitoring), and cryo-TEM, showed that the copolymer strongly interacts with lipid bilayers and effectively stabilizes the nanoparticles. The PNIPAM block retains its thermoresponsive behavior, undergoing a volume phase transition upon heating that leads to polymer collapse. This induces reversible structural changes in both the nanoparticle corona and the internal cubic mesophase, including shrinkage of the aqueous channels. Such modifications can facilitate temperature-triggered release of hydrophilic cargo. Additionally, PDMA-b-PNIPAM-stabilized cubosomes exhibit enhanced affinity for lipid membranes than PF127-based systems, suggesting improved interactions with biological interfaces.

Conclusions and perspective

LLCNPs, particularly cubosomes and hexosomes, have emerged as highly versatile nanocarriers owing to their unique internal organization, structural tunability, and ability to encapsulate diverse classes of bioactive molecules. Over the past decade, and especially in recent years, significant progress has been made in understanding how their internal mesophase, governed by lipid composition and the CPP, dictates key properties such as drug loading, release kinetics, and interactions with biological environments. The transition from lamellar to non-lamellar architectures, as well as

between hexagonal and cubic phases, has proven to be a powerful mechanism for engineering responsive behavior.

Among the different strategies explored, pH-responsive LLCNPs have attracted particular attention due to their ability to exploit physiologically relevant gradients. Notably, enzymatic processes, light, ionic environments, H₂O₂, and thermo-magnetic triggers further expand this landscape by enabling dynamic responses to specific biological or external stimuli. The prominence of pH-responsive systems in the recent literature may reflect their comparatively high level of maturity, particularly considering the growing convergence between LLCNPs and the broader field of pH-responsive mRNA-LNPs [77,79]. By contrast, light-, enzyme-, ionic-, and thermo-responsive LLCNPs remain comparatively underexplored, despite their potential advantages.

In my opinion, pH responsiveness may currently offer one of the most realistic routes toward biological translation, because it relies on endogenous physiological and pathological gradients and does not require an external device. In particular, pH-triggered changes in lipid ionization and packing can be directly coupled to mesophase transitions, providing a mechanistic link between the biological stimulus and nanoparticle function. Enzyme- and H₂O₂-responsive systems offer potentially greater biological specificity, although their effectiveness may depend strongly on the local concentration and spatial distribution of the triggering species. Light-responsive LLCNPs, in contrast, provide excellent spatial and temporal control and are particularly attractive for localized therapies. Although limited tissue penetration remains a constraint for deeply located targets, this limitation can be partially overcome in selected clinical settings through minimally invasive light delivery, including fiber-optic probes, as exemplified by photodynamic approaches for lung and other endoscopically accessible tumors. Importantly, these stimuli should not necessarily be considered as mutually exclusive. Combining endogenous and externally controlled triggers may provide an opportunity to achieve sequential or synergistic responses; for example, pH-responsive LLCNPs incorporating PSs could couple environmentally triggered structural or release processes with light-activated ROS generation. Such multi-responsive systems may offer greater control over where and when therapeutic activity occurs, although their increased complexity also demands rigorous validation to demonstrate that each additional level of responsiveness provides a measurable therapeutic advantage. Thus, future research should move beyond simply expanding the repertoire of stimuli and instead identify combinations of triggers that are biologically relevant, technically feasible, and demonstrably beneficial for therapeutic outcomes.

Remarkably, recent studies have highlighted that internal nanostructure is not only a determinant of physicochemical behavior but also a key factor in biological performance, influencing cellular uptake pathways and intracellular fate. In particular, non-lamellar systems such as cubosomes show enhanced internalization and the ability to partially bypass endosomal barriers, which is highly relevant for the delivery of sensitive therapeutics such as nucleic acids [86].

Despite these advances, several challenges remain. A major limitation is the incomplete understanding of LLCNPs behavior under complex physiological conditions, including the presence of proteins, enzymes, and biological fluids that can alter nanoparticle structure and stability. The formation of biomolecular coronas may significantly affect their *in vivo* performance. Furthermore, the scalability and reproducibility of certain preparation methods, along with long-term stability and toxicity of novel lipid and polymer components, require further investigation.

Future perspectives in this field will likely focus on the rational design of LLCNPs through a deeper integration of structure–function relationships. The ability to precisely tune the CPP and mesophase behavior offers opportunities to develop smart nanocarriers with programmable responses to multiple stimuli. In addition, coupling structural responsiveness with targeting strategies and controlled biological interactions will be essential for improving specificity and therapeutic efficacy.

In this context, polymer-based cubosomes represent an emerging and complementary direction for the evolution of LLCNP technologies. In my opinion, polymer cubosomes are unlikely to simply replace their lipid counterparts but may provide unique opportunities for applications requiring enhanced structural control and multifunctionality. Compared with lipid-based systems, polymeric architectures offer virtually unlimited possibilities for molecular design, including the development of stimuli-responsive and functional nanostructures, improved mechanical and colloidal stability, and a broader range for tuning pore size and transport properties. These advantages may enable more precise control over cargo loading and release behavior. However, lipid-based LLCNPs retain important advantages, particularly their intrinsic biocompatibility, biodegradability, and biomimetic nature, which have contributed to their extensive investigation for biomedical applications. Therefore, polymeric and lipid-based cubosomes should be considered complementary platforms, each offering distinct opportunities depending on the intended therapeutic application.

Although LLCNPs have demonstrated remarkable versatility and responsiveness in preclinical studies, several challenges still hinder their translation into

commercial and clinical products. Recent advances in microfluidic manufacturing have substantially improved the scalability and reproducibility of these systems, representing an important step toward industrial production. However, Good Manufacturing Practice (GMP)-compliant manufacturing processes and large-scale validation remain largely unexplored. In addition, future research should more critically assess the actual advantages of LLCNPs over established nanocarriers such as liposomes and nanoemulsions, particularly with respect to drug loading, release kinetics, stability, and therapeutic performance. Indeed, one of the major weaknesses of the field is the tendency to equate successful structural characterization with demonstrated drug delivery performance. While SAXS and release studies are indispensable for confirming the internal organization and release behavior of LLCNPs, they cannot, by themselves, establish therapeutic relevance. Future studies should place greater emphasis on mechanism-driven biological investigations, quantitative pharmacological evaluation, and direct comparisons with clinically established nanocarriers to substantiate the potential advantages of LLCNP-based drug delivery.

Several priorities should now be addressed to move the field beyond proof-of-concept studies. First, the fate of LLCNP mesophases after administration remains largely unknown, as *in vivo* studies rarely establish whether the original internal nanostructure is preserved, remodeled, or lost under physiological conditions. Second, the absence of standardized stimulus dosimetry, including H₂O₂ concentrations, alternating magnetic-field parameters, and light fluence, makes it difficult to compare responsive systems across studies or to assess whether the applied stimuli are physiologically or clinically attainable. Equally important, the effects of protein corona formation on the internal nanostructure remain poorly understood, despite their potential to alter responsiveness and biological performance.

Addressing these limitations through rational formulation design and standardized comparative studies will be essential to establish LLCNPs as a mature platform for next-generation nanomedicine.

Declaration of competing interests

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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