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Long-acting lipid-based nanomedicines: rethinking from structure-based rational design to *in vivo* fate evaluation☆

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ABSTRACT

Long-acting lipid-based nanomedicines (LaLBNs) aim to sustain therapeutic effect through prolonged exposure and controlled drug release. However, extended circulation does not always translate into improved clinical outcomes. For instance, polyethylene glycol (PEG)-modified liposomes show enhanced pharmacokinetic (PK) parameters such as half-life and area under the curve, yet their benefits, as observed with Doxil®, often fail to meaningfully surpass free doxorubicin. This discrepancy arises because standard PK measurements quantify total drug concentration, which combines both of the encapsulated inactive drug and bioavailable released drug. True therapeutic longevity hinges not on carrier persistence in blood, but on the spatiotemporal pattern of active drug availability at the target site. Therefore, a rational evaluation and understanding of the *in vivo* journey of nanocarriers are essential prerequisites for achieving effective therapy. In this review, we summarize the rational design of LaLBNs and systematically evaluate the *in vivo* fates using an absorption, distribution, metabolism, and excretion framework. We critically assessed existing analytical methods and proposed strategies that integrate both temporal and spatial dimensions to better capture the dynamic fate of LaLBNs. By reframing LaLBNs as active biological entities rather than inanimate carriers, we advocate a paradigm shift from merely prolonging circulation to comprehensively orchestrating the entire delivery process, thereby narrowing the gap between nanocarrier design and therapeutic performance.

Abbreviations: ABC, Accelerated blood clearance; AI, Artificial intelligence; AUC, Area under the curve; CARPA, Complement activation-related pseudoallergy; CMC, Critical micelle concentration; Cmax, Maximum plasma concentration; CD47, Cluster of differentiation 47; Dox, Doxorubicin; DPPC, Dipalmitoyl phosphatidylcholine; DSPC, Distearoylphosphatidylcholine; EPR, Enhanced permeability and retention; FDA, U.S. Food and Drug Administration; Fc, Fragment crystallizable region; HO-PEG, Hydroxyl-terminated polyethylene glycol; IgM, Immunoglobulin M; KCs, Kupffer cells; LaLBNs, Long-acting lipid-based nanomedicines; LBNs, Lipid-based nanomedicines; Lip, Liposomes; LDLR, Low-density lipoprotein receptor; LNPs, Lipid nanoparticles; LSEC, Liver sinusoidal endothelial cells; mPEG, Methoxy-terminated Polyethylene Glycol; mRNA, Messenger RNA; MSPs, Membrane scaffold proteins; PBPK, Physiologically based pharmacokinetic; PK, Pharmacokinetics; PEG, Polyethylene glycol; PEGylation, Polyethylene glycol conjugation; PPE, Palmar-plantar erythrodysesthesia; RES, Reticuloendothelial system; siRNA, Small interfering RNA; SIRPα, Signal regulatory protein alpha; T/NT, Target-to-Non-target Ratio; T_m, Phase transition temperature; TSP-1, Thrombospondin-1; VLP, Virus-like particle.

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1. Introduction

Lipid-based nanomedicines (LBNs), such as liposomes and lipid nanoparticles (LNPs), are widely adopted in clinical practice because of their ability to significantly enhance the bioavailability of poorly soluble or unstable therapeutic agents [1–3]. However, conventional LBNs are susceptible to rapid recognition and clearance by the reticuloendothelial system (RES), resulting in short circulation times [4]. The prevailing evaluation paradigm prioritizes extended blood circulation as a central design objective and efficacy predictor, often employing pharmacokinetic (PK) parameters, such as half-life ($t_{1/2}$), Area under the curve (AUC), and maximum concentration (C_{max}), as the key success metrics [4,5]. To overcome rapid clearance, surface modifications such as PEGylation have been extensively used to substantially prolong circulation time, yielding long-circulating LBNs [6–8]. Within the conventional framework that equates blood drug concentration with bioavailability, a classical perspective has emerged, suggesting that long circulation implies long-acting drugs [9]. Thus, an extended residence in the systemic circulation is presumed to automatically translate into prolonged therapeutic effect. Consequently, long-circulating LBNs are often regarded as long-acting LBNs (LaLBNs) [3,9–11]. Under this rationale, LaLBNs have been promoted to improve drug solubility and safety, and to reshape PK profiles to achieve sustained therapeutic exposure. Such properties are considered highly valuable in fields such as oncology, chronic infections, and gene therapy, where stable drug levels are crucial for efficacy, and a reduced dosing frequency can enhance patient compliance and minimize off-target toxicity [2,12,13].

However, a critical translational paradox remains that markedly improved circulation often fails to yield proportional therapeutic benefits in patients. Despite substantial enhancements in PK profiles, many clinically approved LaLBNs do not demonstrate superior efficacy compared to free drugs (**Table 1**) [14–21]. For instance, PEGylated liposomal doxorubicin (Doxil®), although exhibiting a significantly extended half-life and improved safety profile compared to conventional doxorubicin, did not significantly improve overall survival in some clinical cases [14,15]. Similarly, SPI-077, a PEGylated liposomal cisplatin, showed a prolonged circulation half-life and was well-tolerated. However, it exhibited efficacy in a Phase II clinical trial for non-small cell lung cancer, with an objective response rate of only 4.5% [16]. This disconnection between prolonged systemic exposure and inadequate pharmacological benefits underscores a fundamental gap in understanding the mechanistic relationship among PK behavior, the *in vivo* fate of LaLBNs, and therapeutic output. The current evaluation paradigm heavily relies on the static PK metrics, such as AUC, C_{max}, and $t_{1/2}$, as surrogate markers of efficacy [22]. Although these parameters reflect the retention time of a drug in the body, they suffer from an inherent limitation. In the PK study, the total drug is generally measured without distinguishing between the encapsulated (carrier-associated) and released (free) forms. Only free drugs are pharmacologically active, while the encapsulated cannot engage with biological targets [23]. Thus, conventional PK monitoring captures hybrid kinetics that fail to accurately represent the time-dependent profiles of active agents. This issue is equally critical at the tissue distribution level. Most biodistribution studies focus on the accumulation of total drugs in various organs, overlooking essential questions regarding the drug state (carrier-bound vs. free), its delivery to specific target cells, and how the carrier actively influences biodistribution.

Furthermore, drug carriers should not be viewed as merely inert excipients. They actively participate in and influence the entire *in vivo* journey of the drug, potentially triggering immune responses, causing organ accumulation toxicity, and interfering with intracellular trafficking pathways, all of which profoundly affect drug efficacy and safety [24–28]. Thus, a core challenge in LaLBNs development is the weak correlation between PK and pharmacodynamic (PD) outcomes, which fails to differentiate active from inactive drug forms and overlooks the carrier's active role in shaping drug fate and its intrinsic biological effects. To enhance the clinical translation of LaLBNs, it is imperative to move beyond the long-circulation paradigm and redefine "long-acting" as a system-level outcome that integrates PK stability with carrier-mediated bioactive drug availability. This review aims to systematically analyze the composition and structure of LBNs and their *in vivo* fate, critically assess the limitations of current PK-based evaluation methods, and propose a multidisciplinary framework for the rational design and dynamic evaluation of LaLBNs based on their journey in the body. Only by grounding formulation design in a comprehensive mechanistic understanding of the entire delivery process can we achieve the transition from long-circulating to truly long-acting formulations, ultimately improving clinical outcomes.

2. Lipid-based nanocarrier platforms

The *in vivo* fate and therapeutic efficacy of LaLBNs depend critically on their physicochemical properties, which are largely determined by the choice of nanocarrier platform and its constituent lipid materials. Without the protection of a nanocarrier, conventional small-molecule injectables undergo rapid systemic distribution, enzymatic degradation, and renal clearance or biliary excretion, resulting in short half-lives and necessitating frequent administrations [17,29]. In contrast, LaLBNs

Table 1
Pharmacokinetic-pharmacodynamic comparison of some conventional drugs versus their LaLBNs formulations

Drug	Formulation	Dose	$t_{1/2}$ (h)	AUC	Ref.	Key PD / Clinical efficacy outcomes	Ref.
Doxorubicin	Free doxorubicin	50 mg/m^2	10.4	3.5 (mg•h/L)	[17]	Dose: 50 mg/m^2 (q4w) PFS: 6.8 months OS: 21 months	[15]
	Doxil®	50 mg/m^2	45.9	902 (mg•h/L)		Dose: 60 mg/m^2 (q3w) PFS: 7.8 months OS: 22 months	
Cisplatin	Free cisplatin	100 mg/m^2	~0.38	~5.33 (μg•h/mL)	[18]	Monotherapy response rate: 6-32%	[16]
	SPI-077	100 mg/m^2	99.28	8233 (μg•h/mL)	[16]	No efficacy at 100 mg/m^2 Response rate: 7.1% at ≥200 mg/m^2	
Irinotecan	Irinotecan	100 mg/m^2	5.5-11.5	2.3-6.4 (μg•h/mL)	[19]	In advanced pancreatic cancer, Onivyde®/5FU demonstrated efficacy and safety comparable to FOLFIRI (5FU/leucovorin/irinotecan), but at a cost approximately 30 times higher.	[20]
	Onivyde®(Irinotecan liposome injection)	70 mg/m^2	25.8	1364 (μg•h/mL)	[21]		

Note: PFS, progression-free survival (time from treatment initiation to disease progression or death); OS, overall survival (time from treatment initiation to death from any cause); q4w, once every 4 weeks; q3w, once every 3 weeks; AUC, area under the plasma concentration–time curve; $t_{1/2}$, elimination half-life.

overcome these limitations through multilevel engineering strategies that modulate particle size, surface properties, and environmental responsiveness, thereby effectively reshaping their pharmacokinetic profiles [30–32].

LaLBNs platform, including liposomes, LNPs, micelles, solid lipid nanoparticles (SLNs) and lipid nanodiscs have been extensively investigated in preclinical or clinical settings, demonstrating their considerable versatility and strong translational potential in drug delivery. Each formulation exhibits distinct structural characteristics and functional advantages that directly dictate *in vivo* behavior (**Fig. 1**; **Table 2**) [33]. Several of these systems have achieved notable commercial success (**Table 3**) [12,34–36].

2.1. Liposomes: versatile bilayer vesicles for drug loading

Liposomes, one of the earliest and most clinically successful nanocarriers, are spherical vesicles composed of one or more phospholipid bilayers enclosing a central aqueous core. This unique architecture allows for the simultaneous encapsulation of hydrophilic drugs in the internal aqueous compartment and hydrophobic or amphiphilic agents in lipid bilayers. Several formulations have been approved for clinical usage such as Lipusu® (paclitaxel, for ovarian cancer) [37], Doxil®/Caelyx® (doxorubicin, for ovarian cancer and Kaposi's sarcoma) [14], and Onivyde® (irinotecan, for pancreatic cancer) [38], as well as Vyxeos® (a liposomal co-formulation of daunorubicin and cytarabine for acute myeloid leukemia) [39]. These approvals highlight the versatility and broad therapeutic impact of liposomes, particularly in oncology but also across other disease areas.

Liposomes can be engineered into various structural configurations, including small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles, to customize their biodistribution, drug release kinetics, and cellular uptake for specific therapeutic applications [1]. Their compositional flexibility, biocompatibility, and capacity for both passive and active targeting have solidified their roles as a foundational platform in nanomedicine.

Liposomes are widely surface-modified with PEG to form LaLBNs. Grafting PEG-lipid conjugates onto the liposomal surface creates a hydrophilic, sterically stabilizing corona that minimizes protein adsorption (opsonization) and subsequent recognition by the RES, thereby significantly prolonging the systemic circulation half-life of liposomes. For example, Doxil® exhibits a plasma half-life of over 40 h in human, in contrast to approximately 10 h for free doxorubicin [17,40]. Such extended circulation is a crucial prerequisite for passive tumor targeting *via* the enhanced permeability and retention (EPR) effect [17,41].

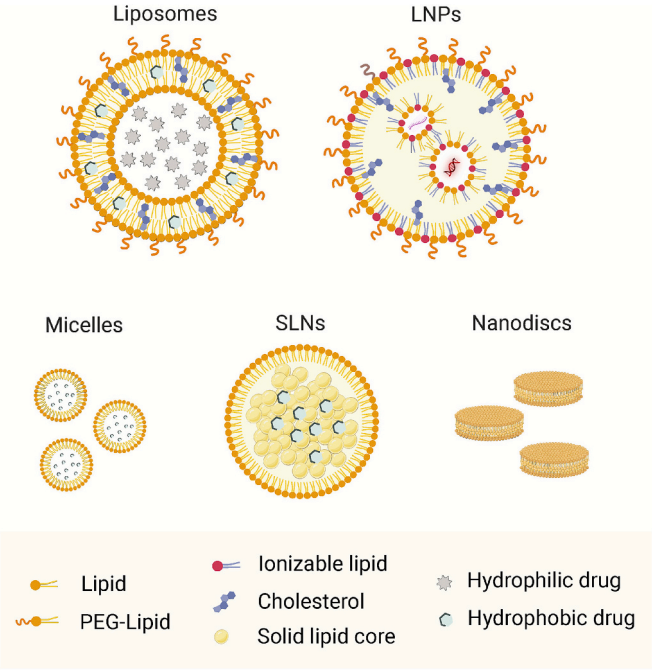


Fig. 1. Structural schematics of the representative LaLBNs, including liposomes, lipid nanoparticles (LNPs), micelles, solid lipid nanoparticles (SLNs) and lipid nanodiscs. Created using biorender.com.

2.2. LNPs: engineered systems for nucleic acid delivery

LNPs have emerged as the leading non-viral platform for nucleic acid delivery. Their clinical impact was first realized with patisiran (Onpattro®), the first FDA-approved small interfering RNA (siRNA) therapeutic, which was authorized in 2018 for the treatment of hereditary transthyretin-mediated amyloidosis [42,43]. This milestone demonstrates the potential of LNPs to enable targeted gene silencing *in vivo*. Subsequently, LNPs played a pivotal role in the rapid development of messenger RNA (mRNA) vaccines, such as Comirnaty® and Spikevax®, during the COVID-19 pandemic, which received emergency use authorization in 2020 [44].

Structurally distinct from conventional liposomes, LNPs feature a dense nonlamellar core composed of ionizable lipids, phospholipids, cholesterol, and PEGylated lipids. This architecture enables the efficient encapsulation and protection of diverse nucleic acid payloads, including mRNA, siRNA, and gene-editing components [45]. Despite these successes, several challenges remain to be addressed. PEGylation can induce immunogenicity, manifested as anti-PEG antibodies, which limits the efficacy of repeated dosing regimens [46]. Moreover, LNPs exhibit pronounced hepatic tropism due to apolipoprotein-mediated uptake, restricting their utility for extrahepatic targeting and resulting in their rapid clearance by RES [47]. Several strategies have been developed to overcome these limitations and achieve prolonged circulation and targeted drug delivery. These include the chemical modification of RNA and novel formulation techniques aimed at enhancing mRNA stability and protection [48–50]. For instance, Su et al. [51] demonstrated that reducing the cholesterol-to-lipid ratio in LNPs diminished liver transfection while enhancing lung-specific targeting.

2.3. Micelles: solubilization with inherent instability

Micelles are a class of nanostructures formed through the self-assembly of amphiphilic molecules driven by hydrophobic interactions in aqueous media [52]. They typically exhibit a core-shell architecture comprising a hydrophobic core and hydrophilic shell, which enables the effective solubilization of poorly water-soluble drugs. Among the various building blocks, polyethylene glycolylated distearoyl phosphatidylethanolamine (PEG-DSPE) is a widely investigated amphiphilic lipid molecule composed of the phospholipid DSPE and a hydrophilic PEG chain [53]. This molecule can spontaneously self-assemble in aqueous environments to form well-defined, monodisperse micelles. The hydrophobic core can encapsulate lipophilic drugs, such as paclitaxel, whereas the PEG shell provides colloidal stability and prolonged circulation characteristics [54]. Currently, micellar systems based on DSPE-PEG are being validated preclinically. In contrast, several polymer-based micelles have received clinical approvals. For example, paclitaxel-loaded micellar formulations, such as Genexol-PM® (using PEG-PLA copolymer) and Zisheng®. These utilize hydrophobic polymer segments to form a stable core, achieving efficient solubilization and tumor-specific accumulation of paclitaxel [55,56].

A long-standing concern regarding micellar systems is their thermodynamic instability in physiological conditions. It has been conventionally thought that intravenous injection leads to rapid dilution below the critical micelle concentration (CMC), causing premature disassembly and drug release [57]. However, recent studies have indicated that micelles can maintain their structural integrity *in vivo* for a

Table 2
Comparison of different lipid-based nanomedicines

Feature	Micelles	Liposomes	LNPs	SLNs	Lipid Nanodiscs
Core Structure	Liquid/Semi-liquid	Aqueous core + lipid bilayer	Solid composite lipid	Solid lipid matrix	Disc-shaped lipid bilayer
Payload Type	Mainly hydrophobic	Hydrophilic, amphiphilic or hydrophobic	Nucleic acids, siRNA, mRNA	Mainly hydrophobic	Mainly hydrophobic
Stability	Moderate to low	High	Moderate	High	Moderate
Circulation Time	Short to moderate	Long (after PEGylation)	Short to moderate	Long (after PEGylation)	Moderate
Preparation Difficulty	Easy	Moderate	Moderate	Moderate	Moderate to high
Clinical Application	Preclinical	Established (Doxil®, Ambisome® etc.)	Emerging (mRNA vaccines)	Established (oral delivery)	Preclinical

Table 3
Representative marketed lipid-based nanomedicines (LBNs)

Type	Company & Brand Name	Active Ingredient	Indication	Approved by	Launch Year
Liposomes	Crucell Berna Biotech - Epaxal	Inactivated hepatitis A virus	Hepatitis A	EMA	1993
	Crucell Berna Biotech - Inflexal® V	Influenza virus strains A and B hemagglutinin	Influenza	EMA	1997
	IDM Pharma - Myocet	Doxorubicin	Metastatic breast cancer	EMA	2001
	Elan Pharmaceuticals - Mepact	Mifamurtide	Non-metastatic osteosarcoma	EMA	2009
	Glaxo Smith Kline - Mosquirix	Recombinant CSP	Malaria	EMA	2021
	Nexstar Pharmaceuticals - Doxil®/Caelyx	Doxorubicin	Ovarian cancer and Kaposi's Sarcoma	FDA/EMA	1995/ 1996
	Sequus Pharmaceuticals - Abelcet®	Amphotericin B	Severe fungal infection	FDA	1995
	Sequus Pharmaceuticals - Amphotec®	Amphotericin B	Severe fungal infection	FDA	1996
	NeXstar Pharmaceuticals - DaunoXome®	Daunorubicin	HIV-related Kaposi's sarcoma	FDA	1996
	Skye Pharm Inc. - Depocyt®	Cytarabine	Neonatal tumor-related meningitis	FDA	1999
	Novartis AG - Visudyne	Verteporfin	Choroidal neovascularization	FDA	2000
	SkyePharm Inc. - DepoDur™	Morphine Sulfate	Pain management	FDA	2004
	Pacira BioSciences - Exparel®	Bupivacaine	Pain management	FDA	2011
	Talon Therapeutics - Marqibo	Vincristine	Acute lymphoblastic leukemia	FDA	2012
	Merrimack Pharmaceuticals - Onivyde™	Irinotecan	Metastatic pancreatic cancer	FDA	2015
	Jazz Pharmaceuticals - Vyxeos®	Daunorubicin and Cytarabine	Acute lymphoblastic leukemia	FDA	2017
	Glaxo Smith Kline - Shingrix	Recombinant Varicella-Zoster Virus glycoprotein E	Shingles and its subsequent neuropathic pain	FDA	2017
	Insmed - Arikayce Kit	Amikacin	Nontuberculous mycobacterial lung disease due to Mycobacterium avium complex	FDA	2018
	Luye Pharma - Lipusu®	Paclitaxel	Ovarian cancer	NMPA	2003
	CSPC Pharmaceutical Group -Duomeisu®	Doxorubicin	Ovarian cancer, HIV-associated Kaposi's sarcoma, and multiple myeloma	NMPA	2022
	CSPC Pharmaceutical Group - Duoenda®	Mitoxantrone	Adult patients with relapsed or refractory peripheral T-cell lymphoma	NMPA	2022
	Jiangsu Hengrui Medicine - Yueyouli@	Irinotecan	Pancreatic cancer	NMPA	2022
	Pfizer/BioNTech - Comirnaty	BNT162b2	Prevention of COVID-19	FDA	2021
	Moderna - mRNA-1273	mRNA-1273	Prevention of COVID-19	FDA	2021
LNPs	Alnylam - Onpattro™	siRNA	Polyneuropathy due to hereditary transthyretin-mediated amyloidosis	FDA	2018
	CSPC Pharmaceutical Group-SYS6006	SARS-CoV-2 spike mRNA	Prevention of COVID-19	NMPA	2023
SLN	Boehringer -Mucosolvan Retard	Ambroxol	Chronic bronchitis	Germany	/

substantial period. For drugs with high plasma protein-binding affinity, such as paclitaxel, the dominant release mechanism is often direct payload transfer from the micelle core to abundant plasma proteins rather than disintegration of the micelle itself [58]. Consequently, although the micellar carrier may remain structurally intact during circulation, its therapeutic cargo can be rapidly released.

2.4. SLNs: a stable delivery platform based on solid lipid matrix

SLNs are spherical nanocarriers composed of biocompatible solid lipids (e.g., triglycerides, fatty acids, waxes) that remain solid at both room and body temperature, with typical diameters ranging from 50 to 1000 nm [59]. Their structure consists of a solid lipid core stabilized by surfactants, which encapsulate active pharmaceutical ingredients in a molecularly dispersed or dissolved state [60]. Unlike aqueous-core liposomes or liquid-core micelles, the solid matrix of SLNs enhances their physical stability and facilitates controlled drug release. Moreover, SLNs lipid components are generally recognized as safe (GRAS), and the system shows low aggregation tendency and minimal drug leakage, supporting administration *via* transdermal, oral, pulmonary, and parenteral routes [61,62].

The sustained-release behavior of SLNs stems from the solid lipid core, which retards drug diffusion and allows release through matrix erosion or surface degradation [63]. Surface modification with stealth

coatings, such as PEG or Pluronic F188, further prolongs circulation by reducing opsonization [64]. These properties make SLNs suitable for long-acting applications in oncology, infectious disease therapy and vaccine delivery [65]. For example, Mishra et al. [66] developed surface-modified SLNs for delivering hepatitis B surface antigen (HBsAg), achieving enhanced cellular uptake and a stronger Th1 immune response compared with soluble HBsAg or mannosylated carriers.

Despite these advantages, challenges remain, including limited drug-loading capacity, potential drug expulsion during storage, and instability due to lipid crystallization. The surfactants used in SLN formulations may also raise concerns regarding cytotoxicity.

2.5. Nanodiscs: a membrane protein-mimetic platform

Nanodiscs are discoidal nanostructures that self-assemble from lipid bilayers encircled by membrane scaffold proteins (MSPs) or synthetic polymers. With a diameter of 10–20 nm and a thickness resembling natural cell membranes (~4–5 nm), they form stable, water-soluble platforms ideal for incorporating membrane proteins, such as G protein-coupled receptors, ion channels, and transporters [67–70]. It was originally developed by Sligar et al. to study cytochrome P450 [68]. Nanodiscs have emerged as promising vehicles for delivering hydrophobic drugs, peptides, and biologics.

The key advantages of nanodiscs include tunable size *via* MSPs or polymer design, and ease of surface modification for targeting or stealth functionalization [67]. Their flat, discoidal morphology promotes unique flow dynamics in the bloodstream. Under shear stress, nanodiscs tumble and rotate, enhancing contact with endothelial cells and favoring vascular wall adhesion [71]. This shape also modulates protein adsorption and reduces immune recognition. For instance, edge-localized PEG can inhibit IgM-mediated complement activation and RES clearance [72]. Nanodiscs can also adsorb apolipoproteins, enabling receptor-mediated transport across barriers, such as the blood-brain barrier [72]. Wang et al. demonstrated that nanodiscs suppress the accelerated blood clearance (ABC) phenomenon upon repeated dosing while retaining tumor-targeting capability of the surface-modified folic acid [73]. Despite these strengths, clinical translation remains challenging because of high MSP production costs, potential immunogenicity, and the need for improved assembly homogeneity and targeting precision.

2.6. Lipid composition as a determinant of nanomedicine performance

The functional performance of LaLBNs stems from the synergistic interplay between their constituent lipids. Each lipid plays a distinct but interconnected role in regulating the assembly, stability, biodistribution, intracellular trafficking, and ultimately, the therapeutic efficacy of LaLBNs [74,75]. Consequently, the rational selection and combination of lipid materials is crucial for optimizing nanomedicine design. The lipid compositions of some clinically approved LBNs are summarized in **Table 4** [14,17,42,76–82].

Table 4
Lipid composition of representative marketed LBNs

Trade name (generic name)	Indication	Lipid composition
Comirnaty® (BNT162b2 mRNA Vaccine)	COVID-19 Prevention	ALC-0315:DSPC: Cholesterol:ALC-0159 (46.3:9.4:42.7:1.6 molar ratio)
DaunoXome® (Liposomal Daunorubicin Citrate)	AIDS-Related Kaposi's Sarcoma	DSPC:Cholesterol (2:1 molar ratio)
Depocyt®	Neoplastic Meningitis	DOPC, DPPG, Cholesterol, Triolein
DepoDur™	Pain management	DOPC, DPPG, Cholesterol and Triolein
Doxil®/Caelyx® (Liposomal Doxorubicin)	Ovarian Cancer, Kaposi's Sarcoma, Multiple Myeloma	HSPC:Cholesterol:DSPE-PEG2000 (56:39:5 molar ratio)
Marqibo® (Liposomal Vincristine)	Acute Lymphoblastic Leukemia	SM:Cholesterol (60:40 molar ratio)
Mepact® (Mifamurtide)	High-grade, resectable, non-metastatic osteosarcoma	DOPS:POPC (3:7 molar ratio)
Myocet®	Combination therapy with cyclophosphamide in metastatic breast cancer	EPC:Cholesterol (55:45 molar ratio)
Onivyde® (Liposomal Irinotecan)	Metastatic Pancreatic Cancer	DSPC:Cholesterol:DSPE-PEG2000 (3:2:0.015 weight ratio)
Onpattro® (Patisiran)	Hereditary Transthyretin Amyloidosis	DLin-MC3-DMA:DSPC: Cholesterol:PEG2000-DMG (50:10:38.5:1.5 molar ratio)
Spikevax® (mRNA-1273 Vaccine)	COVID-19 Prevention	SM-102:DSPC:Cholesterol: PEG2000-DMG (50:10:38.5:1.5 molar ratio)
Visudyne® (Verteporfin)	Choroidal Neovascularization	Verteporfin:DMPC:EPG (1:8 molar ratio)
Xparel® (Liposomal Bupivacaine)	Pain Management	DEPC, DPPG, Cholesterol, Tricaprylin

2.6.1. Structural Lipids

Structural lipids, such as distearoylphosphatidylcholine (DSPC), dipalmitoyl phosphatidylcholine (DPPC), and sphingomyelin, are key components of LBNs. They constitute the bilayer structure of liposomes, form the lipid matrix of LNPs, and contribute to the hydrophobic domains of micelles [83,84]. The selection and ratio of these lipids directly determine the core physicochemical properties of nanocarrier, thereby influencing the formulation stability, drug loading capacity, *in vivo* behavior, and therapeutic efficacy. Saturated phospholipids, which have a higher phase transition temperature (T_m), impart greater structural rigidity to nanocarriers, effectively resisting oxidation and hydrolysis, thereby enhancing long-term stability. For instance, DSPC ($T_m \approx 55$ °C) significantly improves the structural integrity of liposomes and LNPs in physiological environments, reducing passive drug leakage and maintaining vesicle stability, especially in serum-rich conditions [85–87]. In contrast, unsaturated phospholipids have a lower T_m, which enhances membrane fluidity and promotes fusion with the cell membrane [88,89]. However, they are also more susceptible to lipid degradation, potentially leading to premature drug leakage and compromising storage and circulatory stability. Although structural lipids are less commonly employed in micelles, lipid-like polymers with high structural order play a similar role in enhancing kinetic stability and preventing premature disassembly [90].

Notably, the function of structural lipids in LNPs extends beyond passive scaffolding. Kulkarni et al. [91] reported that in empty LNPs, DSPC-cholesterol complexes are primarily localized in the outer layer, whereas in siRNA-loaded LNPs, a portion co-internalizes with the nucleic acid payload. This suggests that structural lipids may actively contribute to payload stabilization and help maintain functional integrity during intracellular transport.

2.6.2. Ionizable lipids

Nucleic acid therapeutics, such as siRNA and mRNA, are characterized by their high molecular weight, negative charge, and susceptibility to nuclease degradation, which hinder their direct penetration through cell membranes and necessitate the development of efficient delivery systems for their administration. Early studies predominantly employed cationic lipids, such as 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and 3β-[N-(N′,N′-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol), to form liposomes or nanocomplexes that electrostatically bind nucleic acids and facilitate cellular uptake [92]. However, these lipids remain positively charged at physiological pH, leading to nonspecific adsorption of plasma proteins, immune activation, and cytotoxicity. These issues often result in compromised *in vivo* stability,

limited safety, and challenges in clinical translation [93].

To overcome these limitations, ionizable lipids (e.g., DLin-MC3-DMA, SM-102 and ALC-0315) were developed. A key design feature of these lipids is the incorporation of a tertiary amine group, which enables smart charge-switching with optimized pKa values (typically ranging from 6.2 to 6.8) and exhibits pH-responsive behavior that is essential for multiple stages of the delivery process [94]. At the neutral pH of blood (~7.4), ionizable lipids remain neutrally charged, minimizing nonspecific interactions with plasma proteins and enhancing systemic stability. Under acidic conditions, such as those within endosomes (pH ~5.5-6.0), the amine groups undergo protonation. This allows electrostatic interactions with negatively charged nucleic acids (e.g., mRNA and siRNA) and promotes endosomal membrane disruption [95]. The protonation of ionizable lipids induces a critical structural transition from a lamellar phase to an unstable nonlamellar phase (e.g., hexagonal HII), which perturbs the endosomal membrane and facilitates the cytosolic release of the payload, thereby addressing a major rate-limiting step in the delivery of nucleic acids [45,96].

2.6.3. Neutral stabilizing lipids

Cholesterol is a classic neutral stabilizing lipid. It intercalates within lipid assemblies, enhancing structural integrity by modulating membrane fluidity, filling packing defects between acyl chains, and promoting membrane fusion. Moreover, cholesterol can be chemically modified to improve drug delivery efficiency. For instance, Zhang et al. [97] constructed an efficient gene transfection system using DC-Chol and DOPE. In another study, Patel et al. [98] demonstrated that replacing 25% and 50% of cholesterol with 7-α-hydroxycholesterol significantly enhanced mRNA delivery efficiency in primary human T cells by 1.8-fold and 2.0-fold, respectively. This improvement was attributed to the modified LNPs, which promoted the increased formation of late endosomes and reduced recycling endosomes, thereby facilitating enhanced endosomal escape and payload delivery.

However, recent studies have revealed a trade-off. Although cholesterol is essential for maintaining carrier stability, excessive cholesterol impedes cellular uptake and intracellular trafficking, ultimately reducing delivery efficiency [45]. For example, in LNPs formulations containing the ionizable lipid KC2, the solubility of cholesterol in the hydrophobic core is limited. Therefore, stabilizing such systems requires reducing the cholesterol content and increasing the proportion of DSPC, underscoring the importance of a tailored design for specific systems [75]. Furthermore, Kawaguchi et al. [99] demonstrated that reducing cholesterol content in mRNA-LNPs can suppress protein expression in the liver, further indicating the influence of cholesterol levels on the systemic circulation and biodistribution of the carriers.

2.6.4. PEG-conjugated lipids

PEG-conjugated lipids are synthetic amphiphiles composed of a hydrophobic lipid anchor (commonly DSPE or cholesterol) covalently linked to a hydrophilic PEG polymer chain. These molecules are integral components in the formulation of LaLBNs, where they are incorporated into the lipid bilayer to confer surface hydration and steric stabilization. The most widely used variant is DSPE-PEG, in which DSPE serves as the membrane anchor, ensuring stable integration into the liposomal or LNPs structure [6,7,32].

The chemical architecture of PEG-lipids critically determines their *in vivo* behavior [6]. Key structural parameters include PEG molecular weight, linker chemistry (e.g., ester, carbamate, or urea bonds), and molar incorporation ratio. The length of the PEG chain influences the thickness of the hydrated steric barrier, while the density of PEG-lipids on the surface affects both colloidal stability and functional interference with biological processes. Notably, PEG-lipids are not permanently anchored; they can gradually desorb from the nanoparticle surface in circulation due to thermodynamic instability, a process that impacts the duration of the stealth effect [100,101].

Furthermore, insufficient PEG coverage can lead to colloidal instability and rapid clearance, whereas excessive PEGylation may sterically hinder cellular uptake [25,101–103]. To address limitations associated with persistent PEG coverage, cleavable PEG-lipid derivatives have been developed. These include pH-sensitive (e.g., hydrazone), redox-sensitive (e.g., disulfide), and enzyme-cleavable (e.g., matrix metalloproteinase substrates) linkers that allow controlled shedding of the PEG layer in response to specific microenvironmental stimuli at target sites [104].

3. Surface engineering for long-acting

Surface engineering is essential for achieving long-acting performance in LaLBNs. Modification of LBNs surface with PEG, polymers, or biomimetic components significantly enhances circulation half-life, colloidal stability, and biodistribution [49]. Rational surface design is critical for minimizing rapid clearance and off-target effects, thereby ensuring a sustained therapeutic efficacy. In this section, we systematically review and compare the mainstream surface engineering strategies based on their core mechanisms, performance advantages, and current limitations, as summarized in **Table 5**. The following subsections provide a detailed discussion of each of these strategies.

3.1. PEGylation: mechanism, optimization, and clinical success

PEGylation, the covalent conjugation of PEG to nanocarrier surfaces, is the most widely adopted strategy in preclinical and clinical nanomedicines for prolonging systemic circulation time. The profound impact of PEGylation stems from its ability to confer stealth properties through several key mechanisms. The hydrophilic and flexible polymer chains form a dense, hydrated steric barrier on the nanoparticle surface. This barrier primarily functions to enhance colloidal stability by preventing aggregation through steric repulsion. Simultaneously, it significantly reduces the nonspecific adsorption of plasma proteins, a process known as opsonization, which is the critical first step leading to clearance by the RES. By effectively minimizing opsonin binding, PEGylation enables the nanocarriers to evade immediate immune recognition, thereby achieving prolonged circulation in the bloodstream [6–8].

PEG is a biocompatible and highly hydrophilic polymer approved by the US Food and Drug Administration (FDA) for use in pharmaceuticals, cosmetics, and food, underscoring its safety and broad applicability [8]. The efficacy of PEGylation is highly dependent on PEG surface density and chain length. Dos Santos and coworkers demonstrated that at a fixed PEG molar ratio of 2%, increasing PEG molecular weight from 2 kDa to 5 kDa significantly improved the AUC of liposomes. However, this benefit diminished at higher PEG densities (5 mol%), indicating an upper limit to the advantages of chain elongation under dense-grafting conditions [105]. Similarly, Ren et al. [106] systematically evaluated the impact of PEG chain length (1, 2, 5, and 10 kDa) and incorporation ratio (5%, 10%, and 20% w/w of total lipid) on the PK profile of liposomes (**Fig. 2**). They confirmed that increasing the PEG length from 1 kDa to 5 kDa enhanced prolonged circulation; however, a reversal occurred at 10 kDa, likely due to the formation of curved micellar structures, which promoted liposome aggregation or structural disruption, thereby shortening the circulation time. Regarding PEG content, liposomes containing 10% and 20% (w/w) of 5 kDa PEG exhibited similar pharmacokinetics, both demonstrating significantly longer circulation than those with 5% PEG. These findings highlighted the formulation-dependent nature of the optimal PEGylation parameters.

The clinical translation of PEGylation has been remarkably successful. The representative approved PEGylated nanotherapeutics include Doxil® for ovarian cancer and Kaposi's sarcoma, which demonstrates a circulation half-life more than 4-times longer than that of free doxorubicin [14]. In addition, Onpattro® (patisiran), an LNP-based siRNA therapeutic that employs PEG-lipid conjugates to avoid immune clearance and facilitate hepatocyte-specific delivery. This enables targeted gene silencing in the treatment of hereditary transthyretin amyloidosis

Table 5
Comparison of PEG and alternative surface modification strategies for LaLBNs.

Modification strategy	Core mechanism	Key performance improvements	Key limitation
Unmodified LBNs	1. No stealth coating 2. Susceptible to opsonization	Reduced parent drug toxicity	1. Rapid RES clearance 2. Short circulation half-life 3. High liver/spleen accumulation
PEGylation	1.Forms a hydrated, flexible PEG corona 2. Sterically hinders protein adsorption	1. Prolonged circulation 2. Reduced macrophage/hepatic uptake	1. Induces ABC phenomenon 2. Impairs cellular uptake & endosomal escape
Cleavable PEG	1. Stealth in circulation 2. PEG sheds at target site	1. Long circulation initially 2. Restores bioactivity after cleavage	1. Cleavage efficiency variable 2. Uncontrolled *in vivo* shedding
Hydroxyl-terminated PEG (HO-PEG)	1. Retains hydration shell 2. Lacks immunogenic termini	1. Long circulation 2. Mitigates anti-PEG immunity	Long-term safety remains under evaluation
Polyvinylpyrrolidone (PVP)	Hydrophilic polymer forms stable hydration layer	1. PEG-like long circulation 2. No ABC phenomenon 3. Inhibits protein adsorption	Long-term clinical safety needs verification
Zwitterionic polymers	Electrostatic hydration for ultra-low fouling	1. Superior antifouling & long circulation 2. Avoids ABC	1. Complex synthesis 2. Limited long-term toxicity data
CD47 / "Self" peptide	Mimics "self" to inhibit phagocytosis *via* SIRPα	1. Suppresses phagocytosis 2. Extends systemic exposure	1. Weak effect alone 2. Minimal PK/liver accumulation improvement
Cell membrane coating	Inherits "self" markers for immune camouflage	1. Extended circulation 2. Reduced immune recognition & RES uptake	1. Complex manufacturing 2. Batch-to-batch variability

[107].

3.2. PEGylation: functional limitations and trade-offs

Despite its well-established success in enhancing the PK profile of nanomedicines, PEGylation introduces a series of intricate functional and immunological trade-offs that substantially compromise the drug delivery efficiency and long-term clinical applicability.

3.2.1. Functional limitations

PEGylation confers beneficial stealth properties; however, excessive incorporation of PEG can compromise liposomal stability and promote the formation of curved micellar structures or other non-lamellar, unstable morphologies [108]. Moreover, PEG exerts a broad inhibitory effect on cellular uptake, impacting not only phagocytic macrophages but also the intended target cells, thereby reducing internalization efficiency. Kuai et al. [109] systematically demonstrated this dose-dependent suppression, observing a progressive decrease in cellular uptake as the molar percentage of PEG-lipid increased from 2% to 10%. The steric hindrance imposed by PEG chains also interferes with the conjugated targeting ligands, restricting their mobility and conformational freedom, thereby leading to a diminished receptor-binding capacity. Hennig et al. [110] reported that PEG conjugation markedly increased the dissociation constant (K_d) of losartan from 1.1 nM to 630 nM, highlighting a substantial reduction in targeting affinity (**Fig. 3A and 3B**). Additionally, the PEG corona significantly impedes endosomal escape, a crucial step for bioactive molecules, such as nucleic acids and proteins, which must reach the cytosol to avoid lysosomal degradation. Song et al. [111] provided evidence that PEGylated liposomes were predominantly trapped in lysosomes without nuclear delivery, whereas non-PEGylated counterparts effectively reached the nucleus.

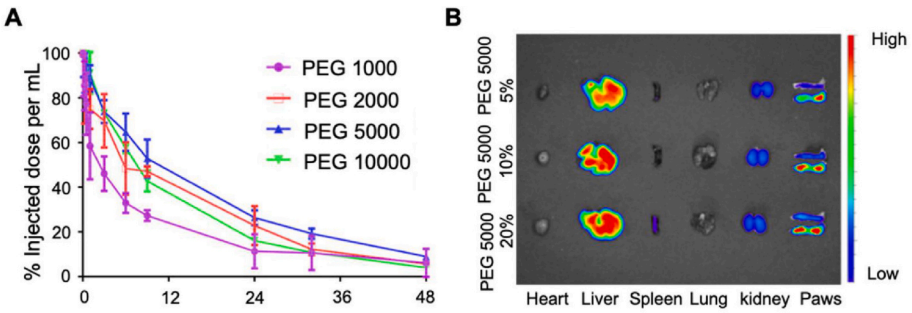
3.2.2. Immunogenic challenges: anti-PEG immunity

Contrary to the initial perception of immunological inertness, PEG is now recognized as an immunogenic agent. Repeated administration of PEGylated nanocarriers can induce the production of anti-PEG antibodies, triggering the ABC phenomenon (**Fig. 3C**) [112–114]. These anti-PEG antibodies bind to the PEGylated surface, promoting opsonization and rapid sequestration by the RES, which markedly reduces their blood circulation time and therapeutic efficacy [115]. In addition to clearance, anti-PEG antibodies activate the complement system *via* the classical pathway. This activation yields potent anaphylatoxins (e.g., C3a, C4a, and C5a), which not only provoke pseudoallergic reactions but also function as opsonins, enhancing immune clearance through complement receptors on phagocytic cells [116]. The high prevalence of pre-existing anti-PEG antibodies in the general population poses a significant concern. As summarized in **Table 6**, the reported seroprevalence has increased dramatically over time. While only about 0.2% of healthy individuals tested positive for anti-PEG IgM in 1984 [117], recent studies (circa 2016) report detection rates ranging from 23% to 72% [118–120]. Notably, a 2019 analysis using highly sensitive assays found prevalence rates exceeding 95% in some cohorts [121]. The exact mechanisms driving this increase remain unclear but are potentially linked to the widespread use of PEG in consumer products (e.g., cosmetics) and pharmaceutical formulations [119,120,122].

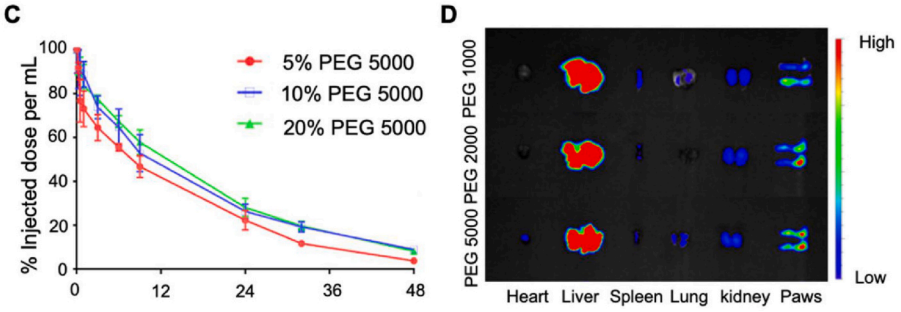
Pre-existing anti-PEG antibodies pose a substantial risk to the efficacy and safety of PEGylated therapeutics. For example, Kozma et al. [123] demonstrated that high pre-existing anti-PEG antibody titers significantly increase the risk of hypersensitivity reactions following mRNA-LNP vaccination in children. In a clinical study of pediatric acute lymphoblastic leukemia, Khalil et al. [124] observed high baseline rates of anti-PEG IgG (13.9%) and IgM (29.1%) among 701 patients receiving PEG-asparaginase; higher antibody levels were correlated with reduced treatment efficacy.

3.2.3. Clinical hurdles

The immunogenicity and functional limitations of PEGylation pose significant clinical challenges to its use. A notable example is the dose-limiting toxicity of palmar-plantar erythrodysesthesia (PPE, or hand-foot syndrome) associated with Doxil® [126,127]. This condition typically presents as painful erythema, desquamation, and ulceration on the palms and soles, frequently accompanied by rashes on the trunk and limbs shortly after infusion. Emerging evidence indicates that this adverse effect is mechanistically linked to complement activation (specifically iC3b deposition) on the surface of PEGylated liposomes. The opsonized liposomes are recognized by neutrophils *via* the CR3 receptor, which internalize and actively transport them across the vascular endothelium into cutaneous tissues. This process leads to localized accumulation of DOX, subsequent keratinocyte damage, and inflammatory responses, providing a direct explanation for the clinical manifestations of PPE and related cutaneous rashes (**Fig. 3D and 3E**) [113]. This pathway not only underlies the dose-limiting toxicity but also underscores a critical trade-off between safety and efficacy in PEGylated



PEG chain length	1 kDa	2 kDa	5 kDa	10 kDa
$AUC_{0\rightarrow t}$(%Dose*h)	823.07 ± 148.48	1485.71 ± 241.12	1705.79 ± 171.00	1373.87 ± 85.98
$T_{1/2}$(h)	11.21 ± 0.26	12.85 ± 1.63	15.83 ± 0.77	11.41 ± 0.08
$MRT_{0\rightarrow t}$(h)	11.09 ± 0.04	14.37 ± 0.33	14.77 ± 0.16	12.00 ± 0.03



PEG content	5%	10%	20%
$AUC_{0\rightarrow t}$(%Dose*h)	1323.12 ± 93.29	1705.81 ± 170.98	1743.58 ± 138.93
$T_{1/2}$(h)	10.52 ± 1.59	15.83 ± 0.77	13.60 ± 0.91
$MRT_{0\rightarrow t}$(h)	13.09 ± 0.59	14.77 ± 0.16	14.57 ± 0.49

Fig. 2. Impact of PEG chain length and grafting density on the *in vivo* fate of liposomes. (A) Pharmacokinetic profiles of liposomes with different PEG chain lengths in mice. (B) Fluorescence imaging of biodistribution in model mice 48 h after injection of liposomes with varying PEG chain lengths. (C) Pharmacokinetic profiles of liposomes with different PEG contents in healthy mice. (D) Fluorescence imaging of biodistribution in model mice 48 h after injection of liposomes with different PEG contents. Adapted with permission from ref [106]. Copyright 2019, American Chemical Society.

nanomedicine design. Preclinical studies suggest that complement inhibition or engineering liposomes with minimized complement activation potential may help mitigate these effects [113]. Furthermore, PEG chains are susceptible to oxidative degradation during storage, which may alter their critical quality attributes and compromise their product performance [128]. The ABC phenomenon, typically triggered after the initial dose, significantly impairs the efficacy of multi-cycle therapies and raises substantial concerns regarding their long-term immunogenicity and safety [129]. Collectively, these factors restrict the applicability of PEGylated liposomes in the management of chronic diseases.

3.2.4. *Strategies to overcome PEG-specific challenges*

In response to these challenges, strategies are being actively pursued. Conventional approaches have focused on optimizing PEG parameters, such as fine-tuning chain length and grafting density to balance stability with reduced immune exposure [130], employing short-chain lipid anchors (e.g., C14 lipids) to facilitate *in vivo* dissociation and reduce RES persistence [131], and designing environmentally responsive, cleavable PEG-lipids (e.g., esterase-sensitive PEG-cholesterol) that shed the PEG layer upon reaching specific physiological environments [132]. Recent advances have expanded the arsenal of available solutions. Tian et al. [114] developed a novel hyperbranched 8-arm-PEG nanostructure that effectively suppressed the ABC phenomenon, attributed to its all-PEG composition which minimizes the presentation of PEG as a hapten on an immunogenic carrier **(Fig. 4A and 4B)**. Building on the insight that human anti-PEG IgM antibodies exhibit pronounced specificity for methoxy-terminated PEG (mPEG), our group devised a strategy utilizing hydroxy-terminated PEG (HO-PEG) as a superior alternative to mPEG. This approach offers a dual advantage, including evasion of pre-existing anti-mPEG antibodies and reduced intrinsic immunogenicity, thereby addressing both pre-existing and induced immune responses [125,133]. We have also pioneered a surface-blocking tactic employing single-chain variable fragments (scFvs). Pre-adsorption of these scFvs onto the PEGylated surface occupied the epitopes recognized by anti-PEG antibodies. Crucially, as scFv lack an Fc region, they block binding without triggering complement activation, thereby providing an effective camouflage **(Fig. 4C)** [134].

3.3. *Non-PEG anti-fouling polymers for stealth nanomedicine*

To address the immunological and pharmacokinetic drawbacks of PEGylation, alternative hydrophilic polymers have been investigated as surface modifiers for nanomedicine applications. These include zwitterionic polymers and PVP, among others, each offering distinct

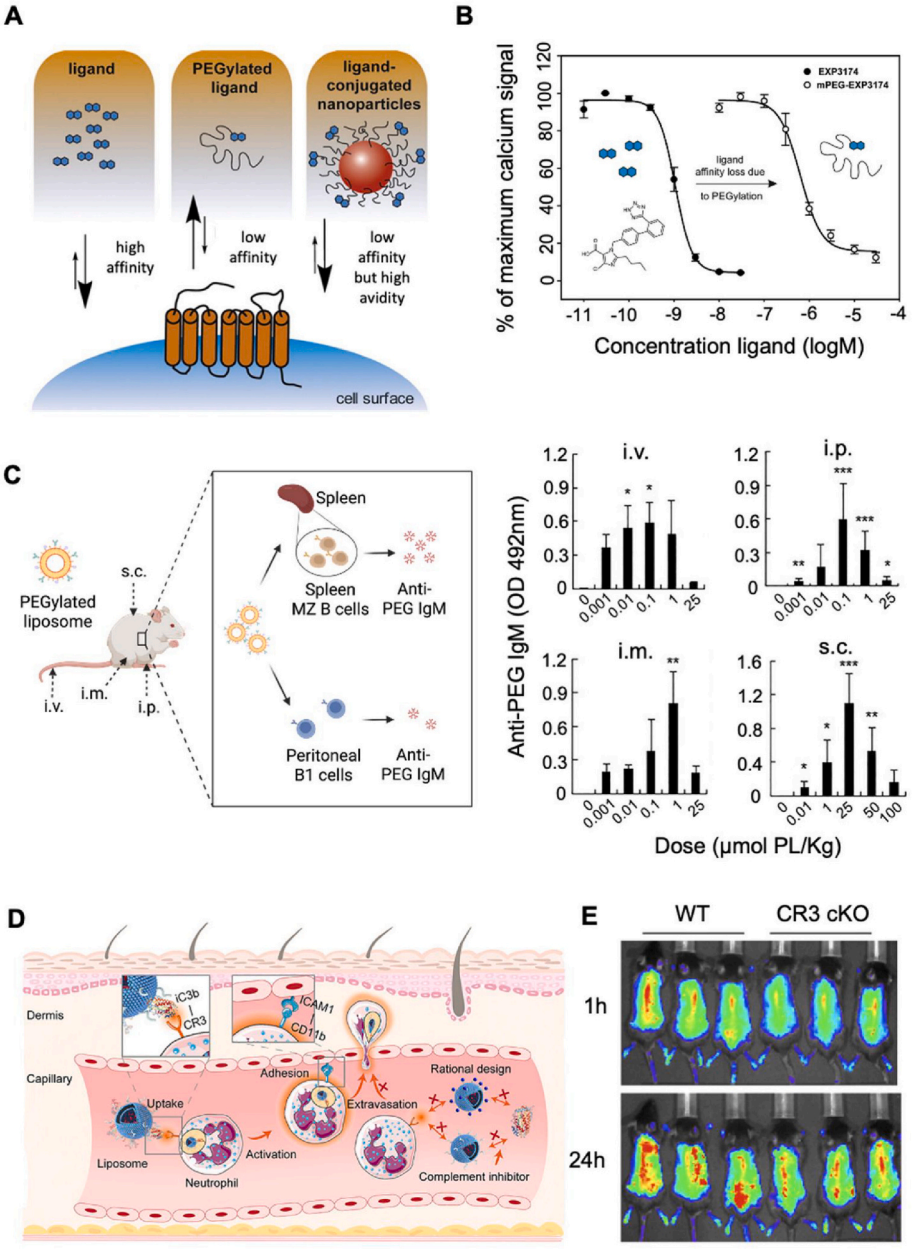


Fig. 3. Limitations associated with PEG modification. (A) PEG modification reduces the binding affinity of targeting ligands; however, multivalent effects can be achieved after carrier modification. (B) The native ligand EXP3174, a selective angiotensin II type 1 (AT_1) receptor antagonist, exhibits high binding affinity with a K_d of 1.1 ± 0.2 nM. Upon PEGylation, the affinity of this small non-peptide ligand is significantly reduced to 630 ± 130 nM, indicating impaired target engagement due to steric shielding. Adapted with permission from ref [110]. Copyright 2014 Elsevier. (C) Anti-PEG IgM is induced in a bell-shaped dose-dependent manner following intravenous, intramuscular, intraperitoneal, and subcutaneous injections of PEG-liposomes. Adapted with permission from ref [112]. Copyright 2023 Elsevier; figure remade using biorender.com. (D) Reciprocal interactions with neutrophils promote cutaneous liposome accumulation. (E) Representative *in vivo* fluorescence images of wild-type and CR3-cKO mice at 1 and 24 h after intravenous injection of sLip/DiD. Adapted with permission from ref [113]. Copyright 2024 American Chemical Society.

advantages in enhancing *in vivo* stability and reducing immune recognition.

PVP has emerged as a promising non-immunogenic polymer for enhancing the long circulation properties of LaLBNs. Torchilin and colleagues [135] reported that incorporation of 10 mol% PVP effectively could completely abolish aggregation induced by polyquaternium salts of negatively charged liposomes. More importantly, a comparative study revealed that PVP-coated nanoparticles maintained consistent elimination half-lives (~20 h) upon repeated administration, whereas PEGylated counterparts exhibited a dramatic reduction in circulation time (from 33.6 h to 1.66 h) due to the ABC phenomenon [136]. Zwitterionic polymers, such as poly(carboxybetaine) (pCB) and poly(sulfobetaine) (pSB), form dense hydration layers *via* electrostatic interactions, effectively resisting protein adsorption and enhancing nanocarrier *in vivo* stability. Li et al. [137] demonstrated that pCB-based surface modification conferred anti-fouling properties comparable to PEGylation, while avoiding the ABC phenomenon and improving tumor accumulation upon repeated dosing. Complementarily, Lin et al. [138] developed PMPC–DSPE conjugates and showed that the incorporation of only 2 mol % DSPE-PMPC into HSPC liposomes prevented aggregation and enhanced colloidal and serum stability, mimicking the natural cell membrane interface. To further advance this field, Zhao et al. [139]

Table 6
Pre-existing anti-PEG antibodys in the population

Population	Detection Method	Conclusion	Year	Ref.
453 healthy volunteers (Japan, Germany, Italy) and 92 allergic patients	Passive hemagglutination assay (PEG-coated RBCs incubated with donor sera)	Anti-PEG antibody prevalence: 0.2% (IgM) in healthy subjects; 3.3% in allergic patients	1984	[117]
28 pediatric patients from ALL-Berlin-Frankfurt-Münster 2000 trials	Serological assay (PEG-RBC agglutination); flow cytometry (PEG-bead immunoglobulin binding)	Among patients with undetectable asparaginase activity post-treatment, 9 were serology-positive, 12 flow cytometry-positive	2007	[118]
377 contemporary and 79 historical serum samples (1970s–1990s)	Competitive ELISA (detection limit: 2–15 ng/mL)	Contemporary samples: 72% anti-PEG Ab-positive; historical samples: ~56% positive	2016	[119]
1504 healthy Han Chinese (756 males, 748 females)	Direct and competitive ELISA	Anti-PEG IgG: 25.7%; IgM: 27.1%; prevalence higher in females	2016	[120]
301 human serum samples (50 obese, 50 diabetic, 200 normal and 1 NHS pool)	ELISA after Tween surfactant removal	Anti-PEG antibody detection rate: 97.5%	2019	[121]
2074 healthy serum samples	ELISA (classified as strong positive [++], positive [+], or negative [–] by absorbance)	Strong positive: 18%; IgM > IgG; higher levels in females and younger individuals	2024	[125]

leveraged the unique adaptive properties of zwitterionic polymers to construct amphiphilic lipid nanoparticles (ZwiLNPs) for efficient siRNA encapsulation and targeted delivery. The design of ZwiLNPs enabled them to adaptively alter surface charge in response to physiological conditions, resulting in remarkable liver-targeting capabilities and improved endosomal escape following cellular internalization. Notably, while these non-PEG polymers demonstrate superior performance in avoiding ABC and maintaining circulation stability, their long-term biological safety profiles require comprehensive evaluation, which is critical for their clinical translation.

3.4. Biomimetic engineering via the CD47–SIRPα "don't-eat-me" pathway

CD47, a transmembrane glycoprotein ubiquitously expressed on the surface of mammalian cells, serves as a key molecular marker of "self" by engaging with signal regulatory protein alpha (SIRPα) in macrophages [140]. This interaction triggers an inhibitory signaling cascade that suppresses phagocytosis, thereby protecting healthy cells from innate immune clearance [141]. Leveraging this biological mechanism, CD47-based biomimetic strategies, particularly the surface display of CD47 or its functional mimetic peptides (e.g., "Self" peptide), have been actively pursued to enhance the *in vivo* longevity of nanotherapeutics [142–144].

Traditionally, long-circulating systems are evaluated by reduced macrophage uptake and hepatic accumulation, metrics established for PEGylated carriers. However, this criterion is less relevant for CD47- or self-peptide-modified nanoparticles, which often show similar total liver accumulation [143]. Tang et al. [142] demonstrated that while self-peptide conjugation did not prevent initial macrophage adhesion, it potently inhibited internalization, indicating that CD47 signaling acted downstream of recognition to block phagocytic execution. *In vivo*, these liposomes were processed more slowly by Kupffer cells (KCs), leading to prolonged systemic exposure despite comparable organ accumulation, demonstrating a kinetic advantage rather than an altered biodistribution. Importantly, the efficacy of the "don't eat me" signal is not uniform across all macrophage phenotypes. Evidence revealed the differential regulation of M1 (pro-inflammatory) and M2 (anti-inflammatory/reparative) subtypes [144,145]. M2 macrophages express high levels of CD36, a scavenger receptor that binds to thrombospondin-1 (TSP-1). The C-terminal domain of TSP-1 can competitively interact with CD47, disrupting the CD47–SIRPα interaction and thereby attenuating the "don't eat me" signal [144]. Consequently, CD47-based evasion is more effective against M1 macrophages, which lack this inhibitory crosstalk, offering a potential avenue for microenvironment-responsive delivery in diseases with polarized macrophage populations, such as tumors and fibrotic tissue. Furthermore, synergistic strategies that combine CD47-mediated active immune evasion with PEG-based passive stealth have been shown to significantly enhance both biocompatibility and delivery efficiency [144].

In addition to peptide-based CD47 display, recent advances have utilized natural cell membrane fusion to engineer nanocarriers with enhanced immune evasion and targeting capabilities [146,147]. This approach involves fusing synthetic liposomes with membrane vesicles derived from specific cell types, such as red blood cells [146], platelets [148], and leukocytes [149], to form hybrid liposomal systems. All these approaches have demonstrated a remarkable ability to improve the PK profiles of drugs *in vivo*.

4. Long circulation but unexpected performance: re-interrogating *in vivo* fate of LaLBNs

Despite decades of research aimed at prolonging the systemic circulation of LaLBNs through engineering strategies, numerous studies have revealed that extended circulation often fails to translate into the anticipated therapeutic benefits, highlighting a significant disconnect between PK optimization and PD outcomes. Therefore, a paradigm shift is urgently needed to redefine LaLBNs from inert drug carriers to active biological entities with dual pharmacological properties. To understand why prolonged circulation does not guarantee efficacy, a holistic understanding of the *in vivo* fate of LaLBNs is required. This requires moving beyond conventional compartmental models to incorporate dynamic blood behavior, tissue distribution and transport, metabolic processing and drug release, clearance pathways, and biological consequences.

4.1. Blood circulation: dynamic remodeling of biological identity

Upon intravenous injection, LaLBNs immediately enter a complex biological environment where their surfaces undergo rapid remodeling, fundamentally dictating the *in vivo* fates (**Fig. 5**).

4.1.1. Protein corona: remodeling biological identity

After injection into the bloodstream, LaLBNs rapidly adsorb biomolecules, such as proteins and lipids, forming a dynamic "protein corona" composed of a tightly bound hard corona and a loosely associated soft corona [150,151]. This layer masks the synthetic surface and dictates subsequent biological interactions. Opsonin (e.g., IgM and complements) promotes recognition and clearance by the RES, whereas dysopsonin (e.g., albumin and lipoproteins) may confer stealth properties and prolong circulation [112,136,152]. Notably, IgM adsorption initiated complement activation *via* the lectin or classical pathway, leading to opsonization and clearance [153]. Moreover, Guan et al. [154] demonstrated that strain-specific differences in plasma protein composition and RES activity in mice affect protein corona formation and the metabolic pathways of liposomes. Furthermore, interspecies

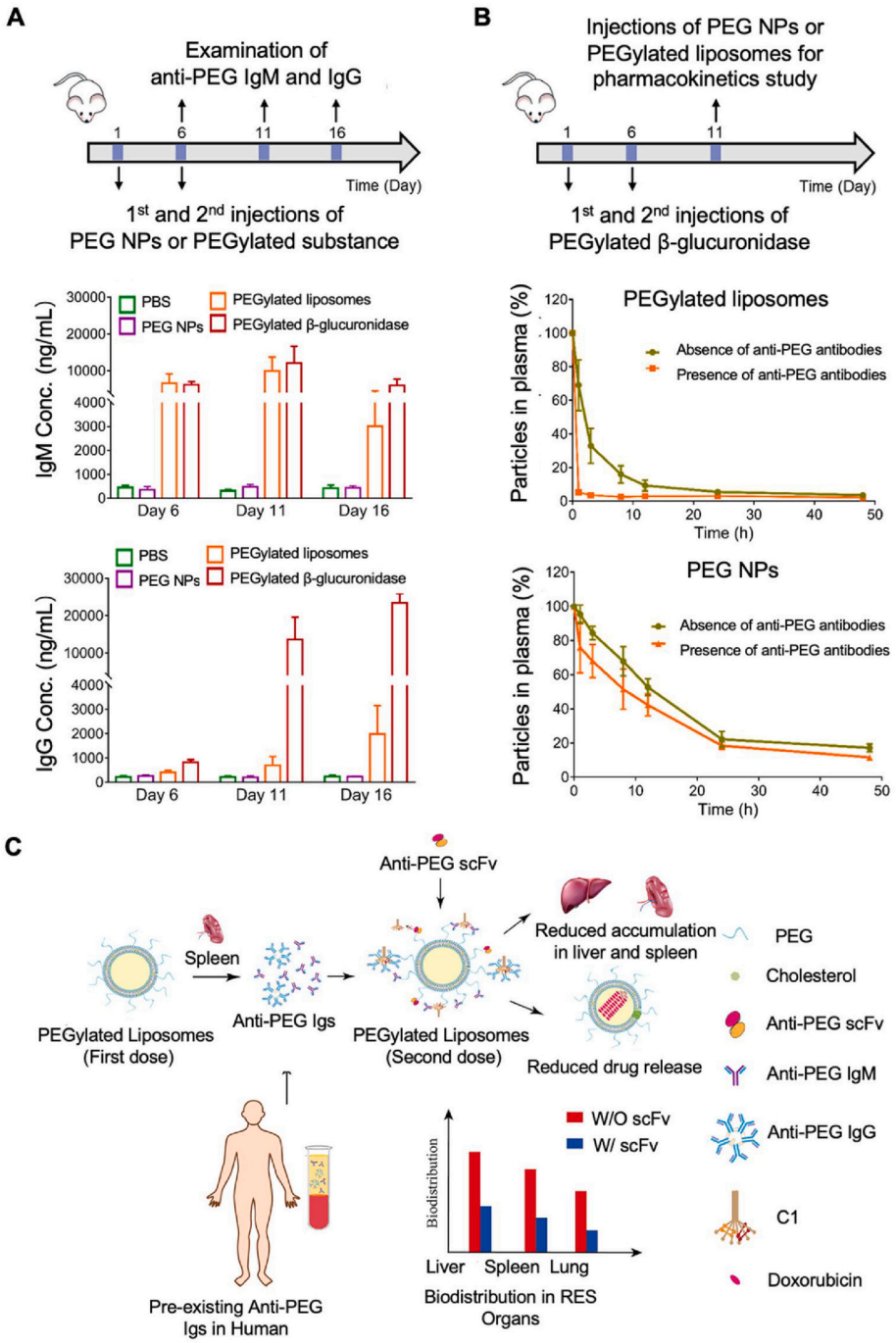


Fig. 4. Amelioration of accelerated blood clearance (ABC) phenomenon through optimization of PEG carrier structure or pretreatment with anti-PEG scFv corona. (A) Kinetics of anti-PEG IgM and IgG production in mice following intravenous administration of PBS, PEG nanoparticles (NPs), PEGylated liposomes, or PEGylated β-glucuronidase on days 1 and 6. (B) Pharmacokinetic profiles of PEGylated liposomes and NPs after pretreatment with PEGylated β-glucuronidase, demonstrating reduced circulation time in the presence of anti-PEG antibodies, indicative of the ABC effect. Adapted with permission from ref [114]. Copyright 2022 American Chemical Society. (C) Anti-PEG scFv corona mitigates the ABC phenomenon in PEGylated nanomedicines. Adapted with permission from ref [134]. Copyright 2021 Elsevier.

variations in the immunoglobulin-complement system influenced drug responses; beagles exhibited higher complement protein levels than rodents, leading to a stronger anti-PEG antibody-mediated ABC phenomenon and a more pronounced pseudoallergy [155].

4.1.2. Surface engineering: a double-edged sword

Surface modification strategies, such as PEGylation, can reduce opsonin adsorption and RES clearance but may induce anti-PEG antibodies and the ABC phenomenon upon repeated dosing [129]. Beyond this well-known dilemma, the incorporation of active targeting ligands can further modulate the immunorecognition of PEGylated systems. Ligands such as folate, RGD peptides, and CDX peptides are commonly conjugated to the distal end of PEGylated lipids (e.g., DSPE-PEG-folate), utilizing PEG as both a stealth layer and a spacer to improve ligand exposure. However, studies have revealed that liposomes functionalized with folate or CDX can adsorb natural IgM antibodies upon intravenous injection. This effect is attributed not to the ligand itself, but to neo-epitopes formed by the structural configuration or clustering of the

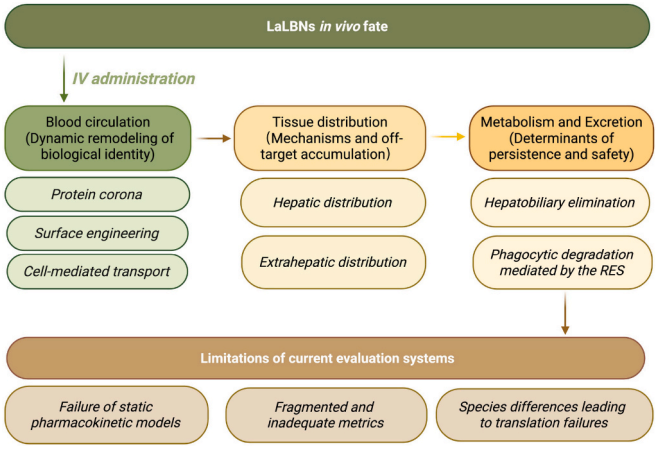


Fig. 5. The *in vivo* fate of LaLBNs the challenges posed to current evaluation systems. Following intravenous administration, LaLBNs undergo a complex, multi-stage process that includes the remodeling of their biological identity in blood circulation (influenced by protein corona formation, surface engineering, and cell-mediated transport), tissue distribution (involving hepatic and extrahepatic accumulation), and ultimately, metabolism and excretion. This dynamic and complex *in vivo* journey reveals the fundamental limitations of current evaluation systems, including the failure of static pharmacokinetic models, the use of fragmented and inadequate metrics, and translational failures arising from species differences.

PEG-ligand complex [156,157]. IgM binding subsequently triggers complement activation and accelerates RES clearance, while simultaneously masking the ligand and impairing its intended receptor-binding capability. Likewise, cRGD-modified PEG-lipids may confer unintended affinity for macrophages, diverting nanocarriers to off-target sites and undermining targeting efficacy [158].

In addition to ligand-specific effects, part of the LaLBNs, especially those with a positive surface charge or high PEG density, can activate the complement system, generating anaphylatoxins (e.g., C3a and C5a) that trigger mast cell degranulation and histamine release, potentially causing complement activation-related pseudoallergy (CARPA), ranging from mild infusion reactions to life-threatening responses [159–161]. Thus, surface modifications aimed at prolonging circulation may pose new biological risks to the body.

4.1.3. Cell-mediated transport

LaLBNs can bind to blood cells, such as monocytes and neutrophils, hijacking their migratory capacity to cross endothelial barriers and enter tissues, including tumors, skin, and inflammatory sites [162]. While this mechanism may enhance drug delivery to certain targets, it can also lead to adverse effects, such as the accumulation of liposomal doxorubicin in the skin, causing PPE [163]. Thus, cell-mediated transport plays a dual role in improving the targeting potential while potentially increasing off-target toxicity. Conventional uptake assays often overlook post-internalization processes, such as intracellular drug release and trafficking, limiting their ability to predict functional targeting, especially for therapeutics requiring specific sub-localization [164].

4.2. Tissue distribution: mechanisms and off-target accumulation

The distribution of LBNs is pivotal to their *in vivo* fate, directly determining their efficacy and toxicity. Owing to their size and inability to freely traverse the intact endothelium, LaLBNs primarily accumulate in specific tissues *via* passive targeting, including the liver, spleen, inflamed regions, and solid tumors.

4.2.1. Hepatic distribution

The liver is the primary site of nanoparticle accumulation for LaLBNs, a phenomenon driven by its unique physiological features, such as highly permeable sinusoidal endothelium, substantial blood supply (representing >20% of cardiac output), and high density of resident macrophages (Kupffer cells, KCs) [165,166]. It is estimated that 30–99% of the administered nanocarriers accumulated in liver. Although well documented, the underlying mechanisms remain complex [166]. Tsoi et al. [167] reported that the velocity of nanoparticles within hepatic sinusoids decreased by nearly 1000-fold compared to that in systemic circulation, significantly prolonging the contact time and enhancing their retention within hepatic tissues. KCs serve as primary clearance cell. Bussin et al. [168] identified several receptor-ligand interactions, such as those involving SR-A, FcγRIIB, HSPGs, SR-BI, and LDL-R, as major contributors to nanoparticle uptake by KCs. Nevertheless, substantial hepatic retention persists even after KCs depletion or receptor pre-saturation, suggesting the involvement of additional, yet uncharacterized, mechanisms (**Fig. 6**) [169]. Mahboubeh Hosseini-Kharat et al. [47] emphasized the role of serum protein interactions, particularly with ApoE, in mediating the liver specificity of LNPs, followed by rapid uptake by hepatocytes.

4.2.2. Extrahepatic distribution

Current strategies for constructing long-circulating nanocarriers aimed at extrahepatic delivery frequently lead to unintended biodistribution and elevated off-target accumulation. For instance, while enhanced PEGylation can prolong systemic exposure, it often results in increased dermal deposition, a key factor underlying clinical manifestations such as PPE [163,170]. More critically, prolonged circulation does not selectively improve targeting; instead, it non-specifically enhances nanoparticle accumulation in multiple non-target organs, including the lungs, kidneys, and bone marrow. This creates a therapeutic dilemma characterized by enhanced targeting, enhanced off-targeting [171,172]. Surface charge further dictates biodistribution patterns. Cationic LBNs, for example, are rapidly cleared by the RES and exhibit pronounced accumulation in pulmonary tissues [93,173]. This explains why some cationic liposomes demonstrate high transfection efficiency *in vitro* but fail *in vivo* due to acute toxicity. These observations underscore that prolonged circulation alone does not guarantee therapeutic safety; rather, it may exacerbate off-target exposure by increasing the duration of nanoparticle presence in the bloodstream.

Moreover, biodistribution profiles of LaLBNs display significant species specificity. While rodent models typically show dominant liver uptake, nonhuman primates often exhibit shifted accumulation toward the spleen and kidneys. These discrepancies reflect inherent interspecies differences in RES activity, lipid metabolism, and cellular receptor expression, which collectively undermine the translational reliability of preclinical data and have contributed to the failure of many long-circulating formulations in clinical trials [17].

4.3. Metabolism and excretion: determinants of persistence and safety

The metabolic and excretion pathways of LaLBNs critically influence their *in vivo* persistence, safety, and functional integrity. Most therapeutic LaLBNs exceed the glomerular filtration threshold (~5–6 nm) and are cleared primarily *via* hepatobiliary elimination and phagocytic degradation mediated by the RES. Clearance begins with RES recognition, followed by phagocytosis, lysosomal transport, and enzymatic degradation, which is catalyzed by lipases and esterase under acidic conditions. The resulting drug molecules and lipid components are ultimately excreted into bile [174].

Using doxorubicin-loaded liposomes (sLip/Dox) as an example [175], our studies integrating cell sorting and drug quantification demonstrated that KCs accounted for the majority of hepatic uptake (~80–90%), while a limited amount of intact PEGylated liposomes could enter the hepatocytes. After internalization by KCs, liposomes are rapidly transported to lysosomes and degraded by acid-dependent lipases and esterase, leading to the release of free doxorubicin and diffuses

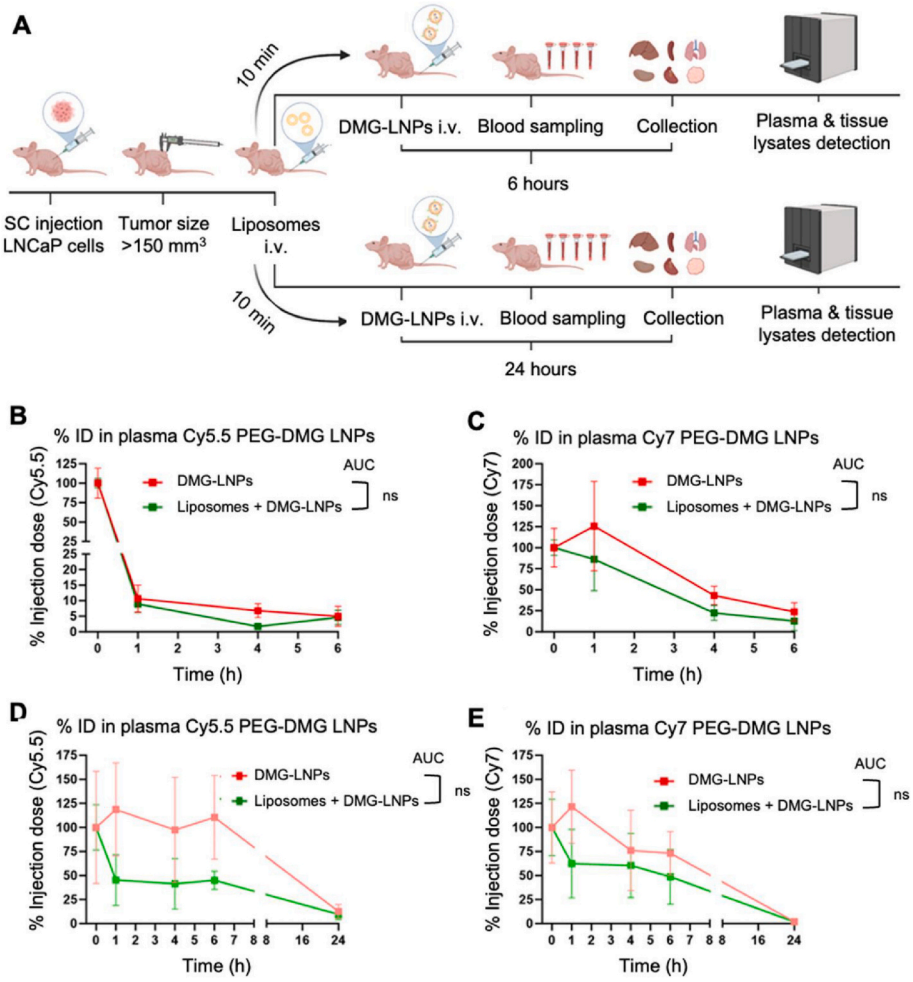


Fig. 6. Liposome pre-treatment does not enhance the circulation time or tumor accumulation of systemically administered PEG-DMG and PEG-DSG siRNA-LNPs in mice. (A) NMRI-nu immunodeficient mice bearing LNCaP tumors were systemically injected with liposomes (360 mg/kg) or PBS (control), followed by i.v. injection of dually labeled PEG-DMG or PEG-DSG siAR-LNPs at a dose of 2.5 mg/kg siRNA. Six hours after PEG-DMG-LNPs and 24 h after PEG-DSG-LNPs administration mice were sacrificed, organs perfused with PBS and tissues were collected for Cy5.5 (siRNA) and Cy7 (lipid) quantification. Plasma fluorescence was quantified for siAR-Cy5.5 (B) and Cy7-DSPE (C) at 1 min, 1 h, 4 h and 6 h after PEG-DMG-LNPs treatment. Plasma fluorescence was measured for siAR-Cy5.5 (D) and Cy7-DSPE (E) 1 min, 1 h, 4 h, 6 h and 24 h after PEG-DSG-LNPs treatment. Adapted with permission under a CC-BY license from ref [169]. Copyright 2025 Elsevier.

into adjacent hepatocytes. This intercellular transfer establishes a distinct zonal distribution throughout the liver lobule, with drug concentrations declining from pericentral to periportal regions, mirroring the functional and metabolic zonation of the liver. Nanocarrier delivery also reprograms the excretion pathway. Doxorubicin that reaches hepatocytes is excreted into the bile largely in its parent form. Subsequent degradation takes place in the intestine under the influence of the gut microbiota, which may contribute to intestinal toxicity and enterohepatic recirculation. Collectively, the sequential processes of slow RES uptake, intracellular drug release, diffusion-mediated redistribution, and prolonged biliary elimination underlie the characteristic slow-in, slow-out pharmacokinetic behavior of sLip/Dox, which stands in sharp contrast to the rapid clearance of the free drug.

This process is modulated by formulation parameters such as lipid composition, PEG architecture, and surface charge, which influence uptake kinetics, degradation rates, and biodistribution. Therefore, a mechanistic understanding of these carrier-specific metabolic and excretory pathways is essential for predicting drug exposure, off-target toxicity, and overall safety.

4.4. Limitations of current in vivo fate evaluation systems

The *in vivo* fate of LaLBNs is highly complex, yet current evaluation frameworks remain largely rooted in paradigms developed for small-molecule drugs, which are inadequate for capturing the dynamic and multi-level behavior of nanomedicines.

4.4.1. Failure of static pharmacokinetic models

Classical compartmental models, which rely on the assumptions of uniform tissue distribution and linear clearance kinetics, fail to adequately describe the complex *in vivo* behavior of LaLBNs. These models do not account for central aspects such as heterogeneous biodistribution, dynamic ligand dissociation, or the evolving composition of the protein corona [176]. Although physiologically based pharmacokinetic (PBPK) models incorporate more realistic organ-level anatomical and physiological parameters, they still fall short of capturing critical dynamic processes, including nanoparticle surface degradation and complement-mediated changes in clearance rates [177,178]. Both model types exhibit considerable limitations in predicting the *in vivo* fates of LaLBNs.

These theoretical and computational limitations are paralleled by the practical shortcomings of *in vitro* release assays. Conventional methods cannot adequately mimic key *in vivo* conditions, such as physiological shear stress, tissue barriers, and immune- and enzyme-rich microenvironments [179]. Moreover, they overlook the time-dependent evolution of the protein corona, which significantly influences drug release and

nanoparticle stability. The poor correlation between *in vitro* release profiles and actual *in vivo* performance misguides the development of long-acting formulations, highlighting a critical gap between experimental models and biological realities.

4.4.2. Fragmented and inadequate metrics

The metrics currently employed, such as the target/non-target (T/NT) ratio, cellular uptake efficiency, and subcellular localization, serve as fundamental and practical tools for the initial characterization of LaLBNs performance. They provide distinct value in quantifying key aspects like targeting specificity and cellular interactions, forming an essential part of the nanomedicine development toolkit. However, when aiming to fully and accurately elucidate the complex *in vivo* fate of LaLBNs, these metrics reveal limitations due to their fragmented and static nature. The T/NT ratio, as an endpoint measurement, is highly sensitive to the sampling time point and fails to capture dynamic processes at the cellular or subcellular level [180]. Although uptake assays are useful for quantifying internalization, they typically overlook critical downstream events such as drug release kinetics and intracellular trafficking. Furthermore, conventional cellular uptake assays often fail to distinguish between nanoparticles that have been genuinely internalized and those merely adsorbed onto the cell surface. This inability to differentiate leads to an overestimation of internalized dose and significantly compromises the accuracy and predictive value of the data for therapeutic efficacy [164]. Similarly, subcellular localization, particularly endosomal escape efficiency, which is crucial for nucleic acid delivery, is frequently neglected in conventional assessments, despite its profound influence on biological activity [181].

Imaging technologies, such as near-infrared fluorescence (NIRF) and single-photon emission computed tomography (SPECT), have become indispensable non-invasive tools for visualizing the distribution of nanomedicines at the whole-body level, providing critical insights for preclinical research. However, their utility is constrained by limitations in spatial resolution, rapid signal attenuation, and limited tissue penetration, which pose challenges for the long-term (weeks to months) dynamic tracking of LaLBNs *in vivo* [182,183]. Consequently, significant evidence gaps remain regarding long-term potential risks, such as lipid metabolic dysregulation, nanoparticle deposition, chronic inflammation, and organ fibrosis. This gap presents a major challenge for establishing safe clinical dosing regimens and therapeutic windows [184].

4.4.3. Interspecies differences as a barrier to translation

Animal models serve as the cornerstone for translating drugs from the laboratory to the clinic, providing an indispensable platform for the initial assessment of the safety and efficacy of nanomedicines. However, the *in vivo* fate of LaLBNs is regulated by mechanisms far more complex than those of traditional small-molecule drugs. Their modes of interaction with biological systems (e.g., plasma proteins, endothelial cells, and immune cells) are fundamentally different, amplifying the impact of interspecies physiological variations and creating significant challenges in cross-species evaluation.

Research has demonstrated these species-specific differences at multiple levels. Guan et al. [154] reported significant strain-dependent variations in plasma protein composition and RES activity in mice, which directly influence liposome protein corona formation and metabolic clearance. Canines exhibit higher complement activity than rodents, potentially exacerbating anti-PEG antibody production and CARPA [155]. Furthermore, the biodistribution of LaLBNs varies considerably between species, uptake is predominantly hepatic in rodents, whereas nonhuman primates show higher accumulation in the spleen and bone marrow. These differences reflect variations in species-specific immune activity and receptor expression patterns, which collectively undermine the predictive value of preclinical models and contribute significantly to the failure of nanoparticle-based therapies in clinical trials.

Beyond interspecies differences, the high degree of inter-individual heterogeneity among clinical patients (e.g., in immune status) likely introduces even greater variability, further complicating the performance prediction and efficacy modulation of nanomedicines.

5. Rational design of LaLBNs enabled by understanding *in vivo* fate

For a long time, the development of LaLBNs has followed a seemingly clear but increasingly inadequate path. Researchers typically construct nanomedicines with ideal physicochemical properties *in vitro*, such as size below 100 nm, PDI under 0.2, and high encapsulation efficiency, followed by animal experiments to validate long circulation and targeting capabilities [185–187]. This linear logic from *in vitro* design to *in vivo* validation essentially treats LaLBNs as inanimate carriers, assuming their behavior can be controlled by a handful of physical parameters. Such an approach largely relies on PK models derived from small-molecule drugs and static distribution assumptions.

However, as systematically discussed in preceding sections, the actual *in vivo* fate of LaLBNs is far more complex and dynamic than can be predicted by these parameters alone. Processes such as protein corona remodeling, surface re-functionalization, off-target accumulation, and inefficient intracellular delivery collectively exhibit nonlinear, time-dependent, and system-coupled characteristics. The limitations of traditional evaluation frameworks, including the failure of static pharmacokinetic models, lack of physiological relevance *in vitro*, fragmented metrics, significant species differences, all point to one fundamental gap. Our understanding on LaLBNs remains at the level of engineered materials, not as dynamic biological entities. As a result, these multi-level complexities demonstrate that the classic, parameter-centric evaluation paradigm is no longer sufficient. This necessitates a fundamental shift toward a new framework where the *in vivo* fate is not an outcome to be passively observed, but a dynamic process to be actively programmed.

5.1. From static parameters to dynamic biological processes

The design of long-acting lipid-based nanomedicines (LaLBNs) has traditionally centered on optimizing individual physicochemical parameters such as particle size, surface charge, or PEG density, under the assumption that these static properties directly determine *in vivo* performance. However, accumulating evidence indicates that the behavior of LaLBNs in the body is not dictated by any single parameter in isolation but emerges from a dynamic sequence of interactions between the nanoparticle's initial structure and the complex biological environment it encounters [4,159,188–192]. For instance, reducing particle size can enhance tumor penetration *via* the EPR effect [189], yet this benefit may be offset by accelerated hepatic clearance [4]. Similarly, high-density PEGylation prolongs systemic circulation time but often suppresses liposome fusion with cell membranes, thereby impairing intracellular drug release and therapeutic efficacy [159,190–192]. These inherent trade-offs highlight a fundamental limitation of the parameter-centric approach: optimizing one property frequently compromises another, leading to suboptimal outcomes.

This realization underscores the need for a shift in design philosophy, from isolated parameter tuning toward a more holistic understanding of the nanoparticle's *in vivo* journey. Rather than pursuing idealized static features, the goal should be to anticipate and leverage the biological processes that govern a carrier's fate, including its circulation, biodistribution, cellular uptake, subcellular trafficking, and payload release. This perspective recognizes that effective delivery arises not from a single attribute but from the integrated outcome of multiple interdependent events. A clear example is liver-targeted LNPs, whose efficient delivery depends not only on passive factors such as size but also on their ability to selectively bind serum ApoE, enabling LDL receptor-mediated internalization into hepatocytes [47]. Likewise, mitigating off-target effects, such as PPE caused by pegylated liposomal

doxorubicin [113,163] or unintended hepatic accumulation and toxicity associated with siRNA-LNPs [193,194], requires moving beyond passive avoidance strategies. Instead, designs must account for active biological interactions, allowing for more precise control over where and how nanoparticles behave in the body.

5.2. From end-point measurement to multidimensional dynamic profiling

The current evaluation system for LaLBNs remains largely fragmented and static, often relying on single-time-point measurements of tissue drug concentration (e.g., 24-h tumor accumulation) or simplified T/NT ratios as key metrics [180]. However, for delivery systems designed for long-acting therapy, initial drug accumulation is less critical than the capacity for prolonged retention and sustained release at the target site. A system exhibiting lower early uptake but capable of forming a stable drug reservoir and gradually releasing active ingredients may possess substantially greater therapeutic potential than carriers exhibiting high initial accumulation but rapid clearance.

To better reflect therapeutic potential, the field must transition from endpoint measurements to multidimensional and time-resolved profiling. This approach emphasizes continuous monitoring of a nanoparticle's behavior across both time and biological scales. It considers not only the quantity of drug delivered to an organ but also the duration of exposure, the specific cell types involved, the efficiency of endosomal escape, the ability to evade lysosomal degradation, and the kinetics of active payload release [181,195].

Temporal resolution spans from the immediate post-injection phase, governing hemodynamic distribution and protein corona formation, to days or weeks of tissue persistence and metabolic processing. Spatial and functional resolution extends from whole-organ imaging down to subcellular localization and molecular activity. For example, in mRNA delivery, even efficient cellular uptake of LNPs results in low protein expression if endosomal escape is inefficient [181,195]. Only by integrating these dimensions can we distinguish carriers that merely accumulate from those that effectively deliver functional payloads over time.

5.3. Balancing fate across scales: toward structure-fate-efficacy mapping

The essence of fate programming lies in establishing a causal mapping relationship from the initial structure of nanocarriers to their *in vivo* fate pathway and ultimately to the therapeutic output. This mapping involves coupled mechanisms across multiple scales and requires the systematic integration of knowledge at the following levels: at the molecular scale, lipid chain saturation influences membrane fluidity and stability; cholesterol content modulates the compactness of the lipid bilayer; PEG chain length and density determine protein anti-adsorption capability and its shedding kinetics [92,105,111,196]; at the nanoscale, parameters such as particle size, morphology, and rigidity directly affect behavior under blood shear stress, endothelial contact probability, and recognition efficiency by the RES [26]; at the systemic scale, the dynamic evolution of the protein corona, complement activation level, and immune cell interactions collectively determine circulation half-life and tissue distribution profiles [24,156]; at the cellular scale, factors including the choice of endocytic pathway, endosome maturation rate, and carrier disassembly kinetics determine whether the drug can be effectively released into the cytoplasm or nuclear target sites [164,181].

Studies have shown that an optimal delivery system design often resides in a dynamically balanced zone under multiple competing constraints. For instance, medium-length PEG chains achieve a superior trade-off between circulatory stability and endosomal membrane fusion capability compared with longer or shorter variants [190]. A moderate positive charge enhances cellular uptake while avoiding excessive complement activation and subsequent rapid clearance [159]. These examples emphasize that the essence of rational design is not maximizing a single metric but rather identifying a system-wide optimum through cross-scale and multi-mechanism synergies.

5.4. Tool ecosystem upgrade

The future development of LaLBNs should transcend traditional material engineering approaches and evolve into a dynamic closed-loop system guided by *in vivo* fate mechanisms and supported by multidisciplinary collaboration. By integrating advanced computational models, high-resolution real-time imaging technologies, and intelligent algorithms, this system aims to shift the LaLBNs design paradigm from empirical trial-and-error and validation-based research to predictive intelligent design.

5.4.1. Biomimetic physiological platforms for predicting long-term in vivo behavior

Conventional *in vitro* models fail to adequately simulate the long-term dynamic processes that LaLBNs undergo *in vivo*, such as sustained release, biodegradation, and immune evasion. Emerging biomimetic physiological platforms, including organ-on-a-chip and vascularized organoid systems, can replicate critical biological barriers and organ-level functions under physiologically relevant conditions over extended time scales. For instance, perfused liver chips can be used to systematically evaluate nanoparticle metabolism, cumulative hepatotoxicity, and tissue retention, thereby establishing more clinically predictive models of hepatic disposition [197–200]. Vascularized tumor organoids facilitate the study of nanoparticle penetration and EPR effects within a simulated tumor microenvironment, providing key translational insights into the design of targeted drug delivery systems [201].

5.4.2. Real-time multi-scale tracking

A comprehensive understanding of the spatiotemporal dynamics of LaLBNs, including biodistribution, release pharmacokinetics, and clearance pathways, is essential for their rational design. Integrated high-resolution imaging and analytical platforms (e.g., PET-MRI coupled with LC-MS/MS and single-particle tracking technologies) provide continuous multi-scale monitoring capabilities from whole-body distribution to subcellular localization [202–205]. Time-resolved data help capture critical kinetic events, such as depot formation at the injection site, target accumulation kinetics, and drug release duration, and identify potential failure modes, such as premature burst release or unintended RES capture. When combined with mechanistic pharmacokinetic models (e.g., PBPK models), these data can inform the reverse optimization of carrier structural parameters, including lipid composition, PEG architecture, and surface charge, to precisely tailor the release profiles and targeting efficiency of the nanocarriers.

5.4.3. Artificial intelligence (AI) enabling predictive and iterative rational design

The design of LaLBNs involves a high-dimensional parameter space encompassing lipid chemistry, surface modifications, particle physicochemical properties, and biointerface characteristics. AI and machine learning offer powerful tools for deciphering the complex nonlinear relationships between formulation parameters and their *in vivo* performance **(Fig. 7)** [206,207]. By training predictive models (e.g., random forest, neural networks, and graph neural networks) on integrated datasets (including composition attributes, *in vitro* assays, and *in vivo* outcomes), AI can effectively correlate design variables (e.g., PEG density and cholesterol content) with key pharmacokinetic endpoints (e.g., AUC, half-life, and release rate), thereby accelerating the screening of optimal formulations [208]. More importantly, AI can drive a closed-loop R&D cycle of prediction, synthesis, testing, and learning. Coupled with molecular dynamics simulations to unravel molecular mechanisms and integrated with PBPK models to predict interspecies differences and clinical translation potential, AI is advancing LaLBNs development from traditional trial-and-error approaches toward predictable, iterative, and high-throughput designs [209].

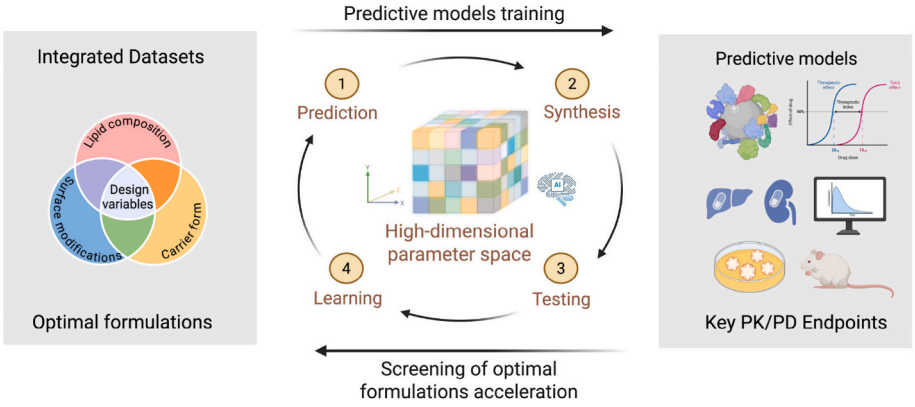


Fig. 7. AI-driven closed-loop of predictive and iterative rational design. Created using biorender.com.

6. Conclusion and outlook

This review critically examines the persistent translational challenges facing LaLBNs, particularly the disconnection between extended circulation time and improved therapeutic efficacy. The prevailing paradigm, which prioritizes prolonged bloodstream residence as a proxy for successful drug delivery, has led to the widespread adoption of pharmacokinetic parameters, such as half-life and AUC, as primary indicators of nanomedicine performance. However, accumulating clinical evidence reveals a concerning paradox: significantly improved PK profiles often fail to correlate with enhanced therapeutic outcomes. This discrepancy underscores the fundamental limitations of the current evaluation frameworks. A central limitation of this approach is the conventional measurement of total drug pharmacokinetics, which conflates the signals from both carrier-encapsulated and free-drug molecules. Standard assays cannot distinguish between intact nanocarriers and released payloads, and thus fail to accurately represent the spatiotemporal distribution of bioavailable drugs. More critically, nanocarriers are not inanimate vehicles but dynamic bio–non-bio hybrid entities that actively interface with biological systems. They modulate protein corona formation, influence immune recognition, direct organ-specific accumulation, and may even induce unintended toxicity or subcellular trafficking barriers. These processes collectively dictate therapeutic success but have historically been underappreciated in LaLBNs design.

Consequently, achieving genuine long-acting efficacy requires a shift in focus from circulation time alone to the integrated optimization of PK stability and site-specific drug release. The rational design of LaLBNs must transition from a narrow emphasis on their physicochemical properties to a system-level approach that prioritizes *in vivo* fate programming. This entails the systematic characterization and deliberate engineering of key biological events post-administration, including serum stability, immune evasion, target tissue accumulation, cellular internalization, endosomal escape kinetics, and controlled drug release.

Looking forward, overcoming the long circulation, low efficiency paradox will depend on building a mechanistic and quantitative understanding of the fate of LaLBNs in living systems. Real-time, *in situ*, and multi-scale technologies capable of resolving the location, state, and biological activity of nanocarriers and their payloads are essential to transform nanomedicine from an empirical practice into a programmable and predictive discipline. Ultimately, the field must evolve from pursuing passive prolonged circulation to achieving active biological targeting and programmed therapeutic action, thereby unlocking the full clinical potential of LaLBNs.

CRediT authorship contribution statement

Yixuan Tang: Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization. **Shan Lu:** Writing – original draft, Methodology, Investigation. **Wanjun Liang:** Methodology, Investigation. **Yujing He:** Methodology, Investigation. **Jifu Hao:** Methodology, Investigation. **Wei Xu:** Writing – original draft, Investigation, Funding acquisition, Conceptualization. **Yuansong Sun:** Writing – original draft, Investigation, Conceptualization. **Changyou Zhan:** Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

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

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