



Review article

The hidden half of lipid nanoparticle manufacturing: Downstream processing

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ABSTRACT

Lipid nanoparticles (LNPs) are the dominant nonviral delivery platform for nucleic acid therapeutics, yet their manufacturing remains complex and difficult to scale reproducibly. Downstream operations, particularly membrane-based unit operations such as buffer exchange and sterile filtration, are central determinants of LNP manufacturability but remain comparatively underrepresented in the academic literature relative to upstream particle formation. This Review demonstrates that downstream processing actively reshapes critical product quality attributes, including particle size, morphology, internal structure, payload distribution, and stability. Each downstream unit operation imposes distinct physicochemical perturbations that collectively determine the final drug product. Many of these changes are formulation-dependent and directly influence biological performance. We identify key knowledge gaps in analytical technologies, mechanistic understanding, and process development practices, and argue that robust LNP manufacturing requires upstream and downstream operations to be designed as a single, coupled process.

1. Introduction

Genetic medicines based on nucleic acid therapeutics, including antisense oligonucleotides (ASO), messenger ribonucleic acid (mRNA), small interfering RNA (siRNA), and plasmid deoxyribonucleic acid (pDNA), constitute a rapidly expanding class of modalities with the potential to treat a broad range of diseases and conditions that are not readily accessible to conventional small-molecule or protein-based therapeutics [1–3]. To exert therapeutic activity *in vivo*, these molecules require delivery vehicles that protect them from degradation, enable cellular uptake, and facilitate intracellular release [4,5]. Both viral vectors and nonviral synthetic carriers have been developed to meet these requirements [2]. Among these approaches, nonviral lipid nanoparticles (LNPs) have emerged as a cost-effective platform for systemic nucleic acid delivery [1,6,7].

LNPs are multicomponent, metastable particles composed of

ionizable lipids, phospholipids, cholesterol, and polyethylene glycol (PEG)-conjugated lipids that form core-shell nanostructures encapsulating the nucleic acid drug substance (DS) [7–10]. LNP manufacturing is divided into upstream and downstream operations, with the upstream part encompassing particle formation and cargo encapsulation (Fig. 1). Downstream processing instead comprises a sequence of unit operations adapted from established biopharmaceutical processing frameworks [11–15], including buffer exchange, conditioning, sterile filtration, and fill-finish. Downstream processing is required to convert the loaded LNP suspension into a drug product (DP) meeting defined specifications for composition, stability, and sterility (Fig. 1). After successful quality control (QC), quality assurance (QA), and batch release (Fig. 1), the LNP DP can be used for preclinical, clinical, or commercial administration to realize its therapeutic intent.

LNPs are highly sensitive to their processing conditions, posing a challenge for reproducible manufacturing. These challenges are

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particularly acute during downstream processing, which remains far less studied than upstream LNP production [16–20]. Unlike conventional drug products, where downstream processing primarily adjusts macroscopic or formulation attributes, downstream operations in LNP manufacturing continue to reshape the distributions of LNP quality attributes. These changes not only shape manufacturability by influencing yield, scale-up robustness, and process control, but also determine the biological behavior of the final DP, including biodistribution, cellular uptake, endosomal escape, and therapeutic efficacy. Yet the impact of downstream unit operations and process development (PD) decisions on LNP quality attributes, biological performance, and manufacturing robustness remains insufficiently understood. This gap reflects the implicit assumption that downstream processing is functionally benign, coupled with practical constraints, including large material requirements, infrastructure and equipment barriers, and throughput mismatches that complicate downstream experimentation. This Review aims to challenge the prevailing upstream-centric LNP view by demonstrating how downstream unit operations reshape the LNP DP, synthesizing current understanding, identifying critical knowledge gaps, and outlining opportunities for future research.

Compared with many mature areas of pharmaceutical process development, downstream LNP manufacturing has benefited less from the traditional interplay between academia and industry (Table 1), as many of its key challenges only emerge under manufacturing-relevant conditions (and with product-specific formulations). Laboratory-scale downstream workflows may generate material that is not fully representative of the final clinical product, meaning that later implementation of manufacturing-scale downstream processes can necessitate additional comparability or bridging studies. Early adoption of scalable downstream operations can therefore improve the translatability of preclinical studies, facilitate technology transfer, and reduce development time and risk.

Table 1

Representative differences between laboratory-scale downstream studies and commercial LNP manufacturing. The comparison highlights the complementary objectives of exploratory academic research and product-focused industrial development.

Aspect	Laboratory studies	Commercial manufacturing
Scale	Milliliters	Liters to hundreds of liters
Manufacturing workflow	Individual unit operations	Integrated manufacturing train
Primary objective	Exploration and mechanistic understanding	Product robustness, reproducibility, and regulatory compliance
Formulations	Model	Product specific (proprietary)
Timelines	Short-duration experiments	Multi-day manufacturing campaigns
Intermediate holds	Limited	Integrated and validated

Recognizing downstream processing as a structural, rather than purely preparative, part of LNP manufacturing reframes how LNP quality attributes, variability, and control are conceptualized in LNP manufacturing. This perspective will become increasingly important as LNP DPs incorporating targeting moieties [21], Selective ORgan Targeting (SORT) LNPs [22,23], liposomal LNPs [24], possibly with combination payloads, further complicate manufacturing, increasing the need for robust downstream process understanding and control.

Importantly, these observations also demonstrate why downstream processing should be considered early in development. Because downstream operations continue to reshape LNP quality attributes, they determine how representative laboratory-produced material is of the final clinical product. Consequently, laboratory-scale downstream workflows that do not translate to manufacturing may generate non-representative material, requiring additional comparability or bridging

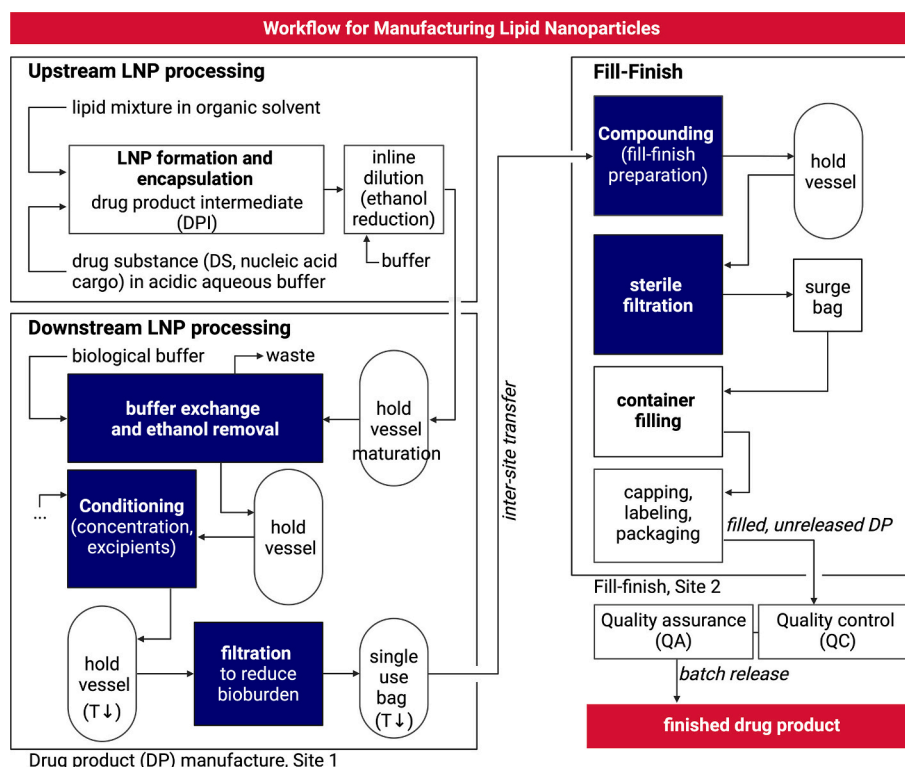


Fig. 1. Representative end-to-end workflow for LNP drug product manufacturing. Upstream particle formation and encapsulation generate a drug product intermediate (DPI), which subsequently undergoes a sequence of downstream unit operations, including buffer exchange, conditioning, bioburden reduction, and, often at a separate facility, fill-finish. Fill-finish comprises sterile filtration, aseptic filling, container closure, and packaging. The overall objective of this workflow is to produce a finished DP that meets predefined quality attributes. Colored unit operations indicate steps discussed explicitly in this review, while unshaded steps are shown for context. Created with BioRender.com.

studies when manufacturing processes change. Early implementation of scalable downstream operations can therefore improve the translatability of preclinical studies, facilitate technology transfer, and reduce development time and risk.

2. LNP manufacturing workflow

LNP manufacturing workflows are structured to deliver a DP that meets defined quality attributes. These attributes span multiple, interconnected domains, including physicochemical attributes such as particle size, size distribution, surface charge (zeta potential), the presence and surface density of targeting ligands, and residual ethanol content. Structural attributes that capture the internal organization and morphology of LNPs, where morphology refers to the presence of a characteristic electron-dense (solid) interior, in addition to aqueous protrusions (also called blebs or biphasic particles), and liposomal structures lacking an electron-dense core [7,25–27], must also be considered. Additional attribute classes include encapsulation-related attributes (cargo loading, encapsulation efficiency, and cargo integrity) and formulation attributes (buffer composition, pH, excipients and cryoprotectants, sterility, bioburden, impurities). Although the workflow described in Fig. 1 is representative, the sequence and duration of downstream operations are ultimately defined during process development by the target quality attributes and formulation-, scale-, and facility-specific constraints.

Upstream LNP processing relies on rapid solvent-driven self-assembly, initiated by mixing lipids in organic solvent (typically ethanol) with nucleic acids in acidic aqueous buffer [7]. This process is readily implemented in continuous mode using microfluidic mixers at small, laboratory scale [7] and jet-based mixers at larger, pilot or manufacturing, scale [28], with the resulting LNP suspension collected in a hold or maturation vessel (Fig. 1). Although particle formation occurs on millisecond-to-second timescales, manufacturing-scale production is governed by substantially longer operational timelines: campaigns require hours of sustained upstream operation at consistent quality, during which LNPs may remain exposed to residual solvents or other destabilizing conditions in the hold vessel.

Downstream processing begins when the LNP suspension exits the upstream mixer and enters the first hold vessel. It typically begins with diafiltration to remove ethanol and accomplish buffer exchange (Section 3). Then, the product concentration is adjusted to the target specification, after which excipients are added (Section 4). Following this, a filtration step is performed for bioburden reduction (Section 5), after which the resulting intermediate drug product (DPI) may be stored under refrigerated (2–8 °C [29]) or frozen (–20 or –80 °C [29]) conditions and, if required, transferred between sites. Immediately prior to fill–finish, the DPI is prepared (e.g., thawed) and, if necessary, subjected to minor compositional adjustments, followed by final sterile filtration as part of the aseptic fill–finish operation (Section 6.1). The filled DP is subsequently stored under controlled conditions until use.

Individual downstream unit operations can span several hours to days and are therefore typically executed as a sequence of batch operations with intermediate holds. During these periods, LNPs may continue to evolve depending on the formulation and storage conditions, resulting in population drift, which we define here as process-induced evolution of the distribution of LNP quality attributes across a particle population.

Commercial manufacturing further emphasizes the importance of intermediate hold times: During process performance qualification (PPQ) for Comirnaty, Pfizer shortened a downstream hold time to meet revised drug product acceptance criteria and subsequently validated the updated hold through cumulative PPQ studies, demonstrating that intermediate holds constitute critical process parameters rather than passive waiting periods [30].

Although exact manufacturing timelines depend on batch size, clinical demand, LNP suspension stability, and facility configuration,

downstream processing is commonly carried out over one or more Good Manufacturing Practices (GMP) shifts (8–16 h), between which material may undergo additional intermediate handling or DPI storage. Consequently, the time from initial LNP formation to DP storage can span several days, approaching one week.

3. Purification and buffer exchange

3.1. Background

LNPs are formed under conditions deliberately chosen to drive rapid self-assembly, typically at acidic pH below the pK_a of the ionizable lipid, promoting protonation and electrostatic complexation with the negatively charged nucleic acid [7]. These formulation conditions are intrinsically incompatible with long-term stability, physiological compatibility, and clinical use, necessitating a buffer exchange step that serves four essential functions. First, residual ethanol must be removed, as it destabilizes LNPs and drives post-formulation structural evolution by increasing lipid fluidity [16,31]. Second, the formulation pH must be neutralized to promote deprotonation of the ionizable lipid, as persistent positive charge is associated with cytotoxicity [32]. Third, ionic strength must be adjusted to achieve near-isotonic conditions with blood plasma, ensuring injectability and minimizing local and systemic adverse effects [33]. Fourth, buffer exchange is accompanied with removal of process-related (low-molecular-weight) impurities such as unencapsulated nucleic acid, free lipids, and small lipid aggregates or micelles. As LNPs are increasingly functionalized with targeting moieties to enable selective delivery [21,22,34–36], buffer exchange also serves to remove excess unbound ligands [37].

Buffer exchange is the dominant transformation step in LNP manufacturing, as it imposes large and coupled shifts in solvent composition, pH, and ionic strength that directly reshape LNP attributes [38–44]. Laboratory-scale studies rely on centrifugal membrane devices or dialysis, whereas manufacturing-scale buffer exchange is implemented using tangential flow filtration (TFF) [45,46]. In practice LNPs are buffer exchanged into one of three biologically compatible buffers: phosphate-buffered saline (PBS) [16,29,47,48], 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-buffered saline (HBS) [47], or tromethamine (Tris)-buffered saline (TBS) [29,47,49]. Buffer selection is non-trivial, as buffer identity has been shown to influence LNP shelf life [29] (Section 4), as well as LNP quality attributes such as particle size, morphology, internal structure, and consequently transfection efficiency [47,50]. Importantly, the purification and buffer exchange step establishes the storage buffer for the remainder of the LNP lifecycle, making buffer selection a critical formulation decision. Recent work further demonstrates that optimal buffer choice may depend on the intended storage strategy: citrate buffer provided the highest transfection efficiency prior to freezing by promoting a more fusogenic internal structure, whereas Tris buffer better preserved particle integrity and transfection efficiency following freeze-thaw [50].

A dilution step is frequently introduced prior to buffer exchange to rapidly reduce the ethanol concentration and kinetically suppress LNP fusion and restructuring [16,39,51–53]. In some cases, such post-treatment have been shown to shrink LNPs [16,52]. Another reason to conduct dilution prior to buffer exchange is to reduce ethanol-induced swelling / plasticization of the membrane [54]. Dilution is straightforward to implement and can be carried out either continuously via inline buffer addition [16,52] or as a discrete operation in a dedicated dilution tank. The dilution step may be intentionally omitted depending on the development strategy and formulation stability, for example to minimize facility time and avoid handling increased process volumes.

3.2. Purification and buffer exchange operation

At small scale, buffer exchange and purification is commonly performed using dialysis and centrifugal ultrafiltration (UF) (Fig. 2). In both

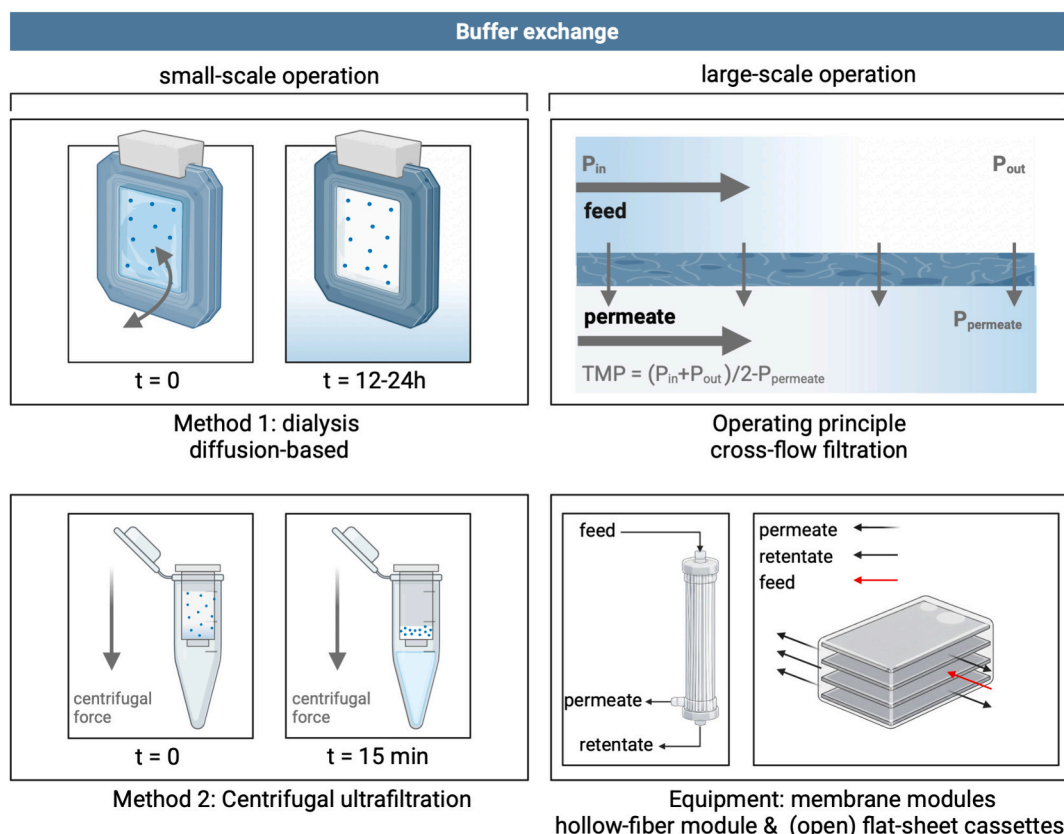


Fig. 2. Small-scale and manufacturing-scale buffer exchange strategies for LNP downstream processing. Left: Small-scale buffer exchange approaches include dialysis using membrane cassettes and centrifugal membrane filtration, enabling low-material operation, but with limited control and scale-up potential. Right: Manufacturing-scale buffer exchange implemented via TFF, in which LNPs are retained and conveyed parallel to the membrane surface while an applied TMP drives solvent and permeable solutes into the permeate. The most commonly used TFF membrane modules (hollow-fiber and flat-sheet cassettes) are also shown. Created with BioRender.com.

cases, process outcomes are governed by a limited set of operational parameters. In dialysis, the sample-to-reservoir volume ratio must be sufficiently large to ensure an effective concentration gradient and to avoid premature equilibration. The membrane molecular weight cutoff (MWCO) and exchange time control the rate of ethanol and buffer removal. In centrifugal UF, applied centrifugal force, membrane MWCO, and loading volume determine permeate flux and the transient concentration at the membrane surface. Both approaches rely on commercial dialysis bags or cassettes, or centrifugal UF tubes with integrated membranes (typically regenerated cellulose, RC), with MWCO of 10–100 kDa [16,55]. Recently, commercial pressure-driven ultrafiltration platforms have also become available at laboratory scale.

For commercial manufacturing, purification and buffer exchange is almost always performed by TFF (Fig. 2), in which LNPs are retained and conveyed parallel to a membrane surface, while an applied transmembrane pressure (TMP) drives ethanol and other permeable solutes into the permeate, enabling controlled buffer exchange and concentration of LNPs [45,56]. TFF-based buffer exchange is performed in diafiltration (DF) mode, in which permeate removal is balanced by continuous addition of fresh buffer to maintain a constant retentate volume [45]. The extent of buffer exchange during DF is quantified by the number of diavolumes (DV), defined as the ratio of cumulative permeate volume exchanged to the (constant) retentate volume.

Performance is largely determined by membrane properties and module type. Membrane permeability, together with transmembrane pressure (TMP), defines permeate flux, while selectivity, typically specified by MWCO, governs solute retention. Usually MWCOs in the 10–500 kDa range [38,39,41,57,58] and membrane materials such as modified polyethersulfone (mPES) or regenerated cellulose (RC)

[38,39,41] are used. Comparisons of commercial RC membranes with identical MWCOs have shown that LNP quality attributes are largely preserved across membrane types, while permeate flux and overall processing time can differ substantially [38]. Subsequent studies indicate that although membrane selection primarily governs flux and process duration, these differences can indirectly influence product quality by modulating the residence time over which structural evolution occurs [39]. TFF is implemented using two module types, large-bore hollow-fiber modules or open (non-screened) flat-sheet cassettes [12] (Fig. 2), both of which are readily scalable. In hollow-fiber modules, the LNP suspension flows through the fiber lumens while permeate passes radially through the fiber walls into the shell side, resulting in well-defined hydrodynamics and moderate shear [59]. Flat-sheet cassettes consist of stacked planar membranes separated by spacer-defined flow channels, through which the retentate flows [59].

Apart from proper selection of the membrane module and properties, TFF performance is governed by a small set of dominant operational parameters, including TMP, number of DVs, crossflow or feed flow rate, and feed concentration or mass load, together with membrane loading, defined as the ratio of processed material to available membrane area, while other variables exert secondary or indirect effects [60]. This illustrates how the effective operating window is strongly conditioned by upstream-defined starting conditions, such as initial ethanol content, total feed volume, and starting LNP concentration. Larger starting volumes increase processing time, while lower initial LNP concentrations necessitate higher concentration factors, thereby increasing exposure time and shear-related stress during TFF. For the extensive buffer exchange typically required in LNP processing, on the order of 5–10 DVs are recommended [15]. Upstream-defined starting conditions can

necessitate an initial UF step to increase effective membrane loading prior to DF, with the initiation of DF commonly selected at a desired balance of flux, buffer usage, and processing time. Beyond these general rules, in practice, TFF operating windows are largely determined empirically during PD.

Although feed (crossflow) fluxes vary substantially across scales, high crossflow velocities are preferred to suppress concentration polarization and fouling. This reduces the buffer exchange per pass, requiring retentate recirculation, increasing residence time and thus cumulative shear exposure [45]. Pump selection is a secondary but non-negligible design choice; peristaltic pumps are used during development for low holdup volumes [15], whereas diaphragm pumps provide lower pulsation and shear, which may benefit sensitive or concentrated LNP formulations.

Some studies distinguish between one-step TFF, in which ethanol removal and pH neutralization occur simultaneously [43,54], and two-step TFF (Fig. 4), in which ethanol is first removed and pH is adjusted in a subsequent step [43]. The latter approach has been reported to yield more stable LNP formulations [43]. However, this benefit must be balanced against increased process complexity and residence time, as prolonged or repeated TFF exposure elevates membrane contact and shear. Depending on the desired concentration, buffer exchange may be implemented as DF-only or integrated directly with other modes of operation, with concentration steps applied either before or after DF [39,58] (Section 4).

3.3. Impact on LNPs

3.3.1. Dialysis

Because dialysis dominates academic LNP buffer exchange workflows [31,44,61], current understanding of buffer exchange effects on LNP quality attributes is largely dialysis-derived. Dialysis-driven ethanol removal and pH increase have been shown to induce LNP-LNP fusion, resulting in size growth, and changes in polydispersity, particularly as the pH crosses the apparent pK_a of the ionizable lipid (Fig. 3) [16,31,40,62,63]. The extent of size growth varies across studies and depends on formulation, processing conditions, and the presence of cargo (Fig. 3) [31,49,51]. Neutralization-driven fusion arises from structural rearrangements triggered by ionizable lipid deprotonation [62,64,65], partially constrained by cargo-lipid electrostatic complexation [31].

pH-induced structural rearrangements are well documented [7], particularly in endosomal escape, where acidification drives lipid reorganization [7,66,67]. In downstream processing, the pH shift occurs in the opposite direction, from acidic formulation conditions toward neutral or near-physiological pH during buffer exchange [40,44,65], which similarly drives active internal restructuring. The structural trajectory depends on both the formulation as well as the final buffer, as shown for drug-free LNPs with small angle X-ray scattering (SAXS) measurements [50]. Time-resolved SAXS measurements demonstrate that structural evolution during dialysis proceeds over hours, with systematic peak shifts indicating kinetically controlled reorganization rather than instantaneous equilibration [40,44]. While initial SAXS profiles differ across studies due to variations in cargo type and upstream processing history [40,44], post-dialysis SAXS features typically become more defined [44,51,65], consistent with reduced ensemble heterogeneity and convergence toward fewer dominant internal structural motifs. Based on the available literature, we hypothesize that post-dialysis LNPs are characterized by the emergence of more dominant lipid phases. For example, in antisense oligonucleotide (ASO)-loaded LNPs, the inverse hexagonal lipid phase becomes more pronounced after dialysis [65], with analogous pH-driven structural convergence reported for mRNA-loaded systems [40,44]. However, given the ensemble-averaged nature of scattering measurements, such observations should be interpreted as indicative of population-level structural convergence rather than definitive assignment of specific internal architectures on a

single particulate level.

Other quality attributes are also affected by buffer exchange. For example, changes in zeta potential have been reported following dialysis (Fig. 3) [51,61,63], reflecting altered surface charge states and lipid ionization. Buffer exchange has further been shown to influence payload distribution and the relative abundance of drug-free LNPs [63,68,69], indicating that population composition can continue to evolve downstream (Fig. 3). Such changes directly alter the amount of cargo delivered per cell, thereby affecting transfection efficiency and potentially immune responses. In contrast, bulk-averaged encapsulation efficiency is largely established during upstream formulation and typically changes only marginally during downstream processing, with modest differences generally compensated for through dose normalization.

3.3.2. TFF

Unfortunately, substantially fewer mechanistic insights are available for TFF-based buffer exchange. The same physico-chemical LNP drivers present during dialysis—ethanol removal, pH neutralization, and ionic strength adjustment—are also operative in TFF. However, TFF operation subjects the LNPs to much greater shear stresses and results in significantly faster buffer exchange than dialysis, and this difference in processing speed is critical [39,40]. Dietrich et al. [39] demonstrated that LNP size increases progressively during TFF and that the extent of growth correlates with total residence time or processing time (Fig. 4). An initially monodisperse LNP population can evolve into a bimodal distribution during TFF; this behavior was reproducible across TFF runs but was not observed during dialysis-based purification [39]. Residence time may explain divergent outcomes reported across TFF studies. He et al. reported minimal changes in size, zeta potential, and morphology during buffer exchange and concentration of DNA-loaded LNPs [54], whereas a silicon-stabilized LNP process using methanol instead of ethanol showed modest but measurable size changes during TFF [58]. The impact of TFF on encapsulation efficiency remains poorly characterized, despite the potential for buffer exchange-induced compositional shifts to weaken lipid-nucleic acid interactions and promote cargo release during prolonged processing.

Another major difference with dialysis is that the mode of TFF operation impacts the LNP properties (Fig. 4) [39,43]. This is important, as TFF operation can be actively exploited as a structuring step rather than a passive purification unit. For example, comparing single-step TFF with two-step TFF shows significant differences in the final product properties [43]. Two-step TFF leads to larger, more stable, and morphologically more uniform LNPs, with a lower fraction of drug-free LNPs [43]. These product quality differences were shown to directly impact the LNPs' in vivo performance [43].

3.3.3. Lipid chemistry and formulation dependence

Beyond operational mode and process parameters, LNP behavior during downstream processing is conditioned by lipid chemistry and formulation composition. This dependence mirrors that observed in upstream formulation and is consistently evident across downstream studies [40,43,49,58,65,68]. Despite this performance variability being widely recognized, it remains poorly documented in the peer-reviewed literature. To date, there is no systematic analysis explaining why certain formulations undergo pronounced size and structural drift during buffer exchange while others remain comparatively stable. This makes dedicated PD essential for each new formulation.

As in upstream processing, deriving generalizable relationships between lipid structure or formulation composition and downstream outcomes is challenging based on the available literature. One contributing factor is that ionizable lipids exhibit distinct pK_a values and/or tail chemistry, such that structural and morphological transitions are triggered at different points along the downstream processing trajectory [40]. Recent molecular simulations suggest that pH-induced deprotonation drives ionizable lipid segregation, phase transitions, and internal reorganization in a lipid-dependent manner, providing a mechanistic

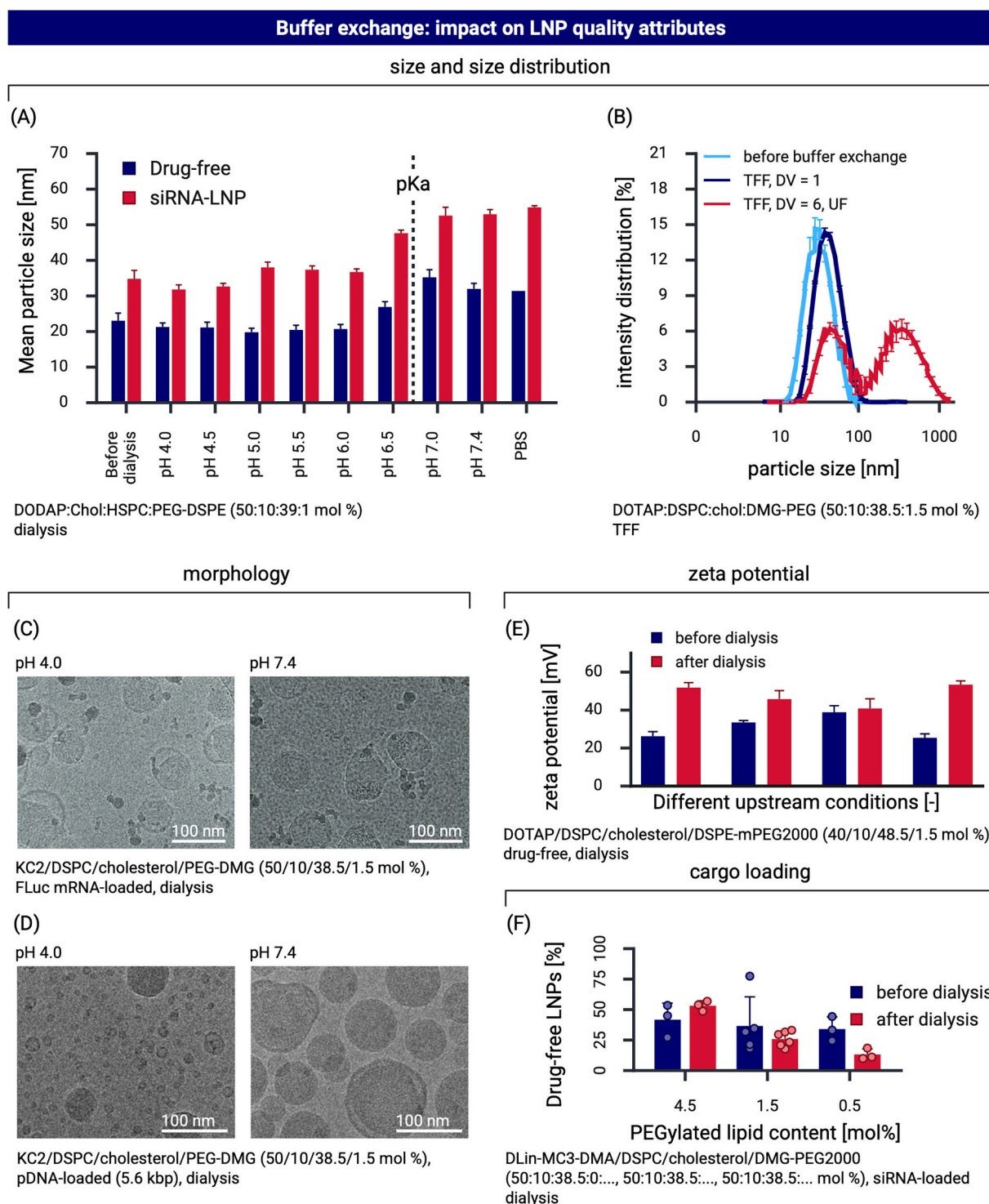


Fig. 3. Impact of buffer exchange on LNP quality attributes. (A) Particle size evolution of drug-free and siRNA-loaded LNPs measured before and after dialysis across increasing pH. Adapted from Terada et al. [31], *Langmuir*, 37, 2021, with permission from the publisher. (B) Intensity-weighted size distributions illustrating differences as the buffer exchange process proceeds from 1 DV to 6 DV. Adapted from Dietrich et al. [39], *Journal of Colloid and Interface Science*, 694, 2025, under a CC BY-NC 4.0 license. (C-D) Representative cryoTEM images before and after dialysis, illustrating dialysis-induced morphological reorganization and particle fusion. Adapted from Cheng et al. [27], *Advanced Materials*, 35, 2023, under a CC BY-NC-ND 4.0 license; and adapted from Kulkarni et al. [62], *Nanoscale*, 11, 2019 with permission from the publisher. (E) Changes in zeta potential before and after dialysis demonstrate buffer- and process-dependent alterations in electrostatic properties. Adapted from Vargas et al. [61], *Colloid and Interface Science Communications*, 54, 2023, under a CC BY-NC-ND 4.0 license. (F) Fraction of drug-free LNPs before and after dialysis, indicating that buffer exchange can alter population composition and payload distribution. Adapted from Li et al. [68], *ACS Nano*, 18, 2024 under a CC-BY-NC-ND 4.0 license. Created with BioRender.com.

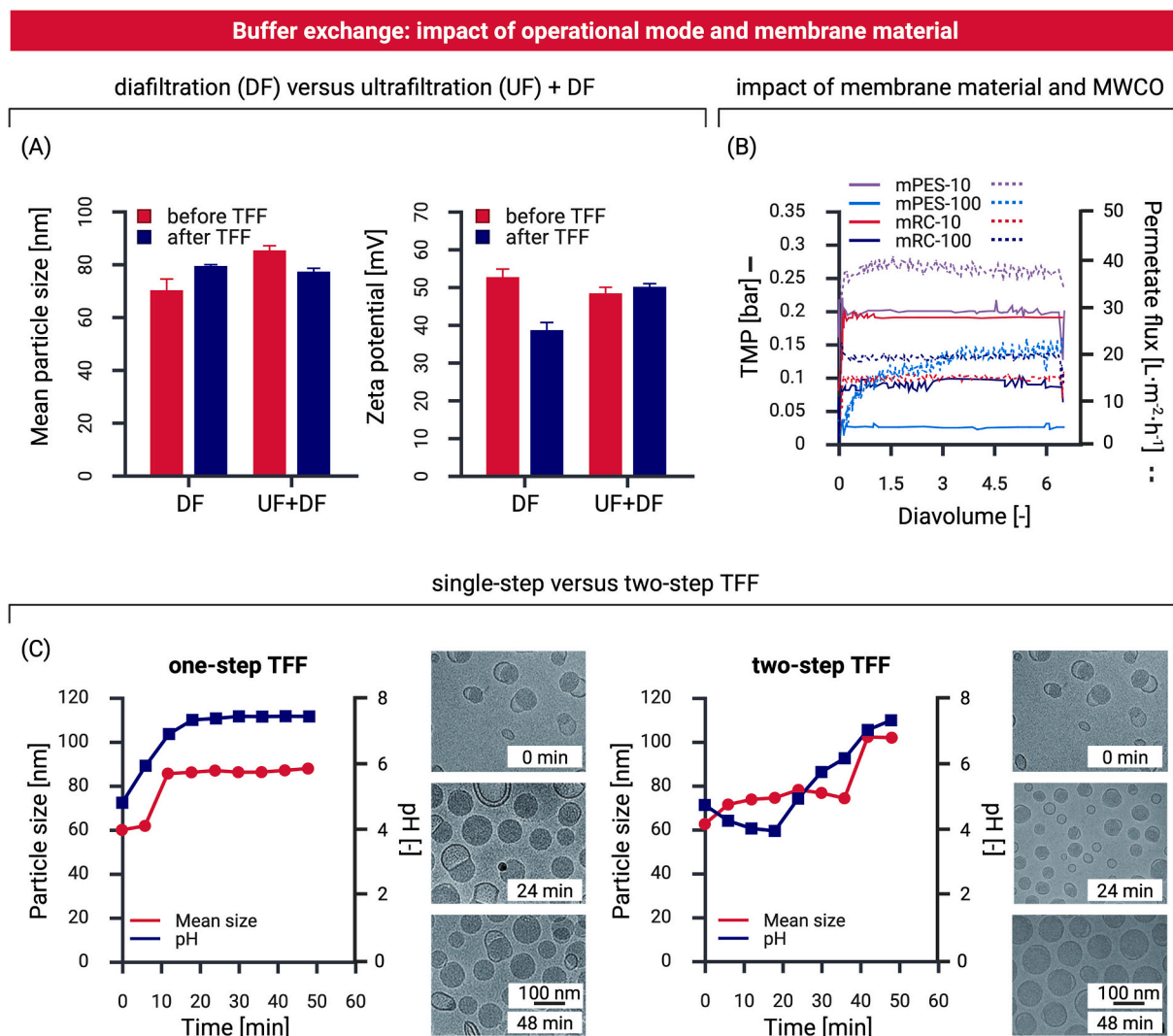


Fig. 4. Impact of TFF operational modes and membrane selection on LNP properties. (A) Comparison of DF alone versus combined DF with UF on mean particle size and zeta potential before and after TFF. Adapted from Saffie-Siebert et al. [58], *Molecular Therapy - Methods & Clinical Development*, 32, 2024, under a CC BY-NC-ND 4.0 license. (B) Influence of membrane material (modified polyethersulfone (mPES) hollow-fiber modules and modified regenerated cellulose (mRC) flat sheet filter modules) and molecular weight cutoff (10 and 100 kDa) on filtration behavior during TFF. Adapted from Dietrich et al. [39], *Journal of Colloid and Interface Science*, 694, 2025, under a CC BY-NC 4.0 license. (C) Comparison of single-step and two-step TFF workflows, in which ethanol removal and pH neutralization are performed either simultaneously or sequentially for a canonical SM-102-based LNP formulation. Time-resolved particle size evolution and representative cryoTEM images. Adapted from Geng et al. [43], *Journal of Controlled Release*, 364, 2023, with permission from the publisher. Created with BioRender.com.

basis for the formulation-dependent behavior [70].

4. Conditioning: concentration adjustment and excipient addition

First filtration, typically using 0.2 μm pore size membranes, is performed for bioburden reduction (BBR) (Section 5), after which a formulation conditioning step is often introduced to establish the final product concentration and to add excipients. If required, buffer concentration or osmolality can also be adjusted at this stage.

Reducing the LNP suspension concentration can be readily achieved by dilution, whereas increasing concentration is more challenging. Dialysis does not permit small-scale concentration increases, in contrast to centrifugal membrane filtration and spin centrifugation–dialysis that couple buffer exchange and concentration [71]. Microfluidic ion concentration polarization further enables electrokinetic LNP concentration [72]. At larger scale, concentration increases are achieved by operating TFF in UF mode [45], either before or after buffer exchange (Section 3). However, elevated LNP concentrations can destabilize formulations and

increase downstream complexity by promoting aggregation, population drift, and membrane fouling.

The addition of excipients is primarily intended to kinetically suppress time-dependent LNP changes (see Section 6.2), including structural rearrangement, fusion, and cargo leakage. Industrial stabilization strategies increasingly employ nonionic surfactants, such as poloxamers and polysorbates [73], analogous to approaches used with many monoclonal antibody products. Such stabilizing excipients are expected to become increasingly important as efforts to reduce PEGylated lipid content to mitigate anti-PEG immunogenicity [74,75] also reduce the steric stabilization of LNP suspensions. Recent academic work demonstrates that excipients may serve functions beyond stabilization. Incorporation of selected saponins into mRNA-LNPs enhanced endosomal escape and modulated vaccine immunogenicity, without affecting its bulk properties [76].

Another approach to suppress kinetic quality attribute drift is through freezing; however, this requires the presence of cryoprotectant agents (CPAs), which mitigate freeze-induced stresses and prevent irreversible damage during freeze-thawing [48,77,78] (see Section 6.2).

The most common excipients added are sugar-based CPAs, in the 0–10 w/v% range. Optimization of CPA identity and concentration (typically 10 mg/mL [79,80]) must be considered [81], as CPA inclusion increases operational sensitivity to aseptic control due to increased bioburden growth, increased viscosity, and foaming. There are also biological implications, as demonstrated by Kim et al. [48]. Following storage at -20°C , formulations containing 10% (w/v) sucrose elicited antibody responses indistinguishable from freshly prepared LNPs, whereas formulations containing 0% or 5% (w/v) sucrose showed reduced immunogenicity [48].

5. Bioburden reduction and sterility assurance

5.1. Background

Although alternative administration routes are under investigation [82–84], parenteral delivery remains dominant in clinical and commercial LNP applications. Consequently, most LNP products are classified as injectables [85] and must be manufactured and filled under sterile conditions in compliance with EU GMP Annex 1 [86] and FDA guidance on sterile DP and aseptic processing [87]. Although sterilization strategies such as autoclaving [88] and gamma irradiation [89] have been explored for nanoparticle formulations [90], they are largely incompatible with current commercial LNP manufacturing due to risks of structural and functional degradation. Sterile filtration therefore remains the dominant approach for microbiological control while preserving product quality [91].

Membrane filtration in LNP manufacturing serves distinct roles across stages. All starting materials are sterile-filtered prior to upstream use. Following TFF-based buffer exchange and conditioning, the LNP suspension is typically subjected to a first filtration step for BBR before transfer into a sterile single-use bulk container or bag [92], constituting a DPI suitable for intermediate storage and, if required, inter-site transport. A separate sterile filtration immediately precedes aseptic fill–finish (Section 6.1) and constitutes the regulatory terminal sterilizing step.

Sterile filtration is a binary size-exclusion step in which sterility assurance depends on full volumetric challenge through a micron-scale pore size membrane operated in dead-end (normal-flow) mode [91]. Particles exceeding the nominal pore size, typically 0.20–0.22 μm , are retained and excluded from the final DP. In practice, redundant filtration trains are commonly employed, with a higher-capacity prefilter (e.g., 0.8 μm) upstream of the sterilizing-grade membrane.

Despite limited study in LNP contexts, sterile filtration is a critical, irreversible terminal step with no corrective downstream options; failure results in batch rejection. As a binary size-exclusion process, material either passes or is retained, making it a potential point of high-value product loss through filter retention. Elevated aggregation can drive fouling, clogging, and premature termination, amplifying yield loss and manufacturing risk.

5.2. Sterilization operation

The most detailed publicly available insights originate from collaborative studies between Zydne's group and Moderna [93–96]. Across these studies, sterile filtration performance is shown to be governed by a limited set of input parameters: LNP concentration, applied pressure (inlet and differential), and membrane characteristics such as area and pore architecture. Together, these parameters determine membrane loading, permeate flux and its decay, product recovery, and volumetric throughput. Device-level factors, including flow geometry and distribution within the module, further modulate shear and fouling, with flow maldistribution or geometric restrictions reducing effective membrane utilization and accelerating performance loss [91].

Membrane selection is a critical determinant of sterile filtration behavior [97]. Essentially all commercial sterilizing-grade filters

provide complete retention of microbiological contaminants [97], but their performance with respect to product recovery, filtration capacity, and flux can vary substantially, particularly for LNPs with mean sizes approaching the nominal membrane pore size. Academic studies predominantly employ modified polyethersulfone (PES) membranes [38,93] at LNP concentrations around 1 mg/mL [94]. Membrane pore size distribution, not membrane chemistry, is the dominant determinant of sterile-filtration performance for LNPs [94]. Manufacturing-scale performance is typically extrapolated from laboratory membrane-disk experiments using capacity and resistance models, despite differences in module geometry relative to pleated cartridge filters used at scale [91].

Fig. 5 shows the filtrate flux increases with volumetric throughput for different TMPs [93]. Analysis of the resistance–throughput relationship reveals an initial pressure-independent permeability, followed by resistance curves that rise more slowly at higher TMPs, ultimately resulting in increased overall capacity with increasing applied pressure [93,94]. The reduction in fouling rate and a concomitant increase in filter capacity at higher TMP is atypical for most biologic sterile filtration processes, in which compressible fouling layers exhibit monotonic increases in hydraulic resistance with increasing TMP [94]. Such unexpected behavior has also been reported for liposomal systems [98], suggesting that such effects reflect an intrinsic property of soft lipid assemblies under confinement rather than an anomaly. Further pressure-step filtration experiments combined with membrane surface microscopy (Fig. 5) showed that sterile filtration fouling is dominated by pore blocking within the 0.2 μm sterilizing layer rather than the 0.8 μm prefilter [93], indicating that the primary foulants are sub-micron in size.

The payload-dependent behavior that LNPs exhibit may lead to differences in sterile filtration performance, particularly at high TMPs: mRNA-loaded LNPs are mechanically soft and reversibly deformable, tolerating compression to approximately one-third of their original height prior to rupture [99], whereas pDNA-loaded LNPs are stiffer and fail via irreversible rupture under mechanical stress [100]. For mRNA-loaded LNPs, a deformation-dominated filtration regime has been proposed, in which soft, amorphous lipid deposits intrude into membrane pores above a critical TMP, leading to non-classical pressure-dependent reductions in filtration resistance and increased apparent filter capacity [94]. Such behavior further implies the possibility of transient pore entry or passage of LNPs with effective sizes exceeding 0.22 μm , as observed in syringe-filter studies [39].

In practice, sterile filtration processes are designed to operate well below membrane failure thresholds, such that filter failure during manufacturing is rare. Nevertheless, post-filtration integrity testing is essential to verify that the final sterilizing filter remained intact throughout product filtration and maintained its performance under process-relevant conditions.

LNP formulations containing particles ≥ 200 nm exhibit reduced recovery during sterile filtration due to membrane fouling via adsorption or deposition [91]. Accordingly, a narrow and uniform particle size distribution improves filtration performance [19]. For 100–150 nm LNPs prepared by Moderna [25,101], Zydne's group reported yields $>96\%$ with stable passage over time despite partial pore blocking [93]. In contrast, other studies have reported more variable yields (72–96%) [39], though this may be related to the filtration operation mode (manual operation).

5.3. Impact on LNPs

Sterile filtration preferentially removes larger particles and aggregates, but it may also reshape the administered LNP population. Given the established influence of particle size on biodistribution and biological performance, such changes could also alter *in vivo* behavior [101,102]. In one study, LNPs between 100 and 150 nm exhibited minimal changes in Z-average diameter following sterile filtration [93].

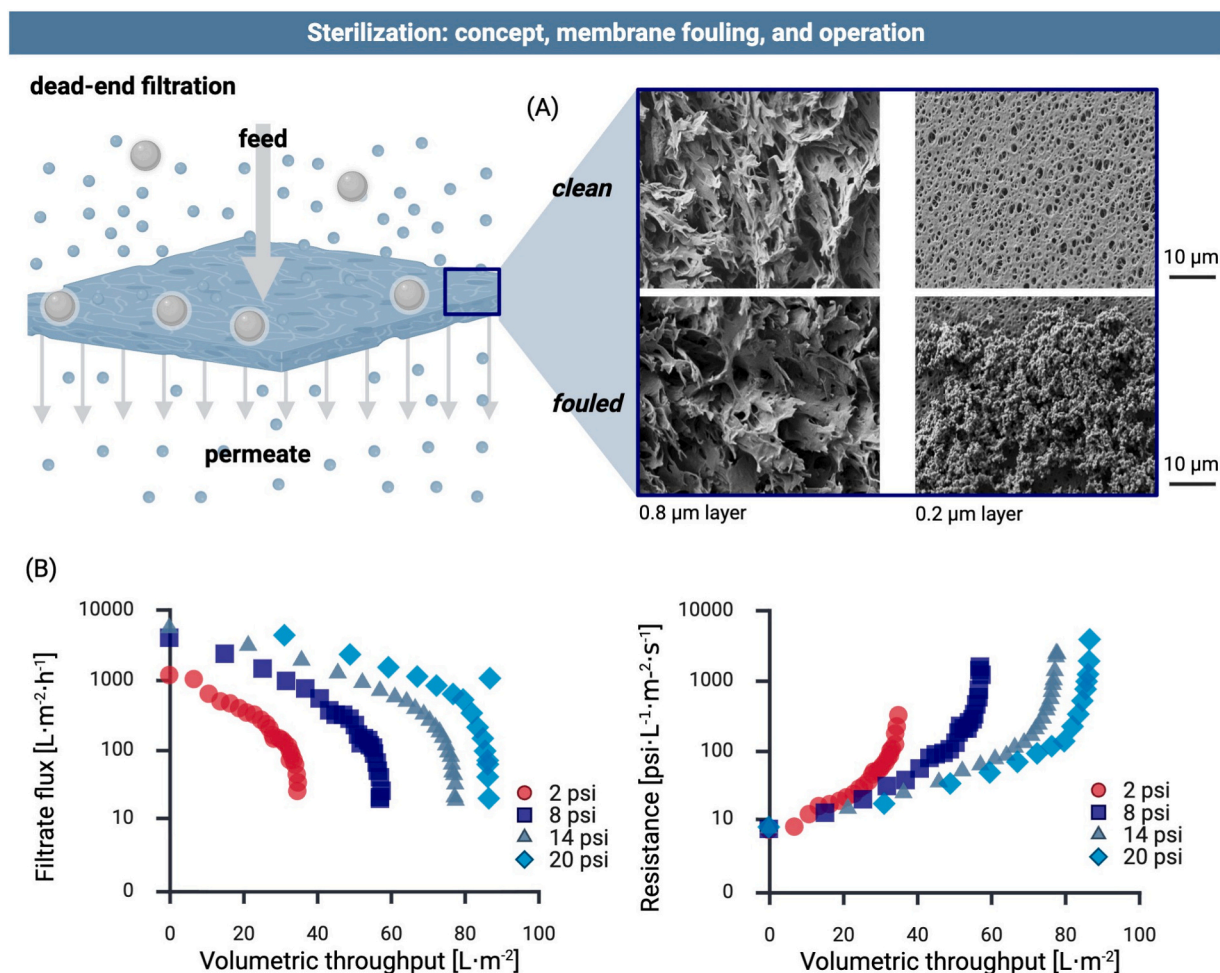


Fig. 5. Sterile filtration as a downstream operation for LNPs. (A) Schematic of dead-end sterile filtration, illustrating particle retention at the membrane surface and the potential for surface deposition and pore blockage. Representative scanning electron micrographs show clean versus fouled membrane surfaces after LNP processing. (B) Effect of TMP on filtrate flux decline and resistance buildup as a function of volumetric throughput during sterile filtration of LNPs. Despite flux decay, high filtration capacities were achieved, with overall LNP yields exceeding 96% across all tested pressures. Adapted from Messerian et al. [93], *Biotechnology and Bioengineering*, 119, 3221–3229. Adapted with permission. Created with [BioRender.com](#)

In contrast, others [39] reported substantial shifts in the underlying size distribution that were only weakly captured by mean and averaged size metrics.

6. Final drug product preparation

6.1. Fill-finish

Fill-finish refers to the final aseptic operations that convert the DPI into the final DP in its container-closure system, ready for storage, quality control testing, batch release, and use. As the terminal downstream stage, fill-finish is regulatory-critical: perturbations introduced here directly affect product quality and are irreversible. Line losses must be minimized to prevent high-value product wastage. Fill-finish represents a mechanically intensive stage of LNP manufacturing, where equipment design, fluid dynamics, and material handling dominate process performance.

For LNP DPs, dosing requirements combined with small fill volumes (0.25–0.5 mL/vial [29,79,80]) can render sterile filtration, rather than the container filling operation itself, the throughput-limiting step during fill-finish, particularly in high-volume applications such as commercial vaccine production. In practice, sterile filtration is commonly decoupled from container filling using an intermediate surge bag.

When frozen DPI is used, fill-finish begins with controlled thawing,

followed by aseptic mixing to restore homogeneity. Cold-chain handling may induce surface condensation on equipment, elevating bioburden risk if uncontrolled. Final sterile filtration (Section 5) precedes aseptic filling into the final containers. For low-viscosity formulations such as LNP suspensions, fill volumes tend to closely match targets; however, sugar-containing formulations increase foaming during handling and filling, potentially compromising accuracy. Commercial filling further employs overfill strategies to ensure complete dose recovery. Filling is followed by stoppering, sealing, and crimping to establish the closed container-closure system [103].

Following aseptic filling and container closure, commercial LNP products undergo 100% visual inspection to detect particulate matter and container defects prior to batch release. During commercial production of LNP DPs, white visible particles were occasionally detected during fill-finish [30,104]. Although they were considered to pose minimal risk because they were product-derived and did not have any toxicological concerns, they were removed through routine visual inspection [30,104].

Fill-finish of LNPs has received limited attention in academic literature, largely due to restricted access to aseptic filling equipment and GMP infrastructure outside industrial settings. Current understanding is therefore inferred indirectly from studies on shear and interfacial stresses and their impact on LNP stability [105–107], though translation to manufacturing practice remains uncertain. In practice, equipment

selection—particularly pumps—is biased toward low-shear designs to minimize mechanical destabilization. Aseptic requirements [86] further impose sterilization of direct- and indirect-contact components and adherence to first-air principles governing equipment layout and operation.

6.2. Storage stability

Stabilization of the final LNP DP and the encapsulated DS to retain long-term therapeutic activity is a major focus of both academic research [7,48,108–111] and regulatory submissions [30,104]. Commercial LNP DPs continue to rely predominantly on sub-zero storage for long-term stability (> 10 weeks) [29]. Repeated freeze-thaw cycles expose LNPs to mechanical stresses and freeze concentration, which alters the local physicochemical environment and generates osmotic stresses [112]. Freeze-thawing has been shown to induce structural reorganization and particle aggregation/fusion, accompanied by changes in critical quality attributes including particle size, morphology, internal structure, and cargo retention [47,50]. In severe cases, substantial losses in encapsulation and transfection efficiency have been reported, although the magnitude of these effects is highly formulation- and buffer-dependent [47,50]. Interestingly, freeze-thaw cycles have also been reported to induce bleb formation [47,50], which has been associated with increased therapeutic activity [16,27] and may enhance freeze-thaw stability by partially sequestering mRNA within aqueous bleb compartments, thereby reducing disruptive lipid-RNA interactions [113]. Rather than solely representing a source of instability, freeze concentration can be exploited as a formulation strategy by incorporating functional molecules into LNPs during freeze-thaw to enhance endosomal escape and mRNA delivery [112].

Efforts to extend shelf life and reduce cold-chain dependence [7,77,108,110,114] increasingly focus on stabilization technologies that convert liquid suspensions into dry powders that do not require sub-zero handling. This is typically achieved through water removal using freeze-drying [115,116], spin-freeze-drying [77], thin-film freezing [117], or spray drying [118]. These approaches must be integrated aseptically, typically after filling, before final container closure, while minimizing vial-to-vial variability. In practice, all of these technologies face the same central challenge: preserving the supramolecular organization of the LNP so that both physicochemical and biological properties are recovered upon reconstitution. Different LNP formulations respond differently to stabilization strategies [48], suggesting that also the optimal drying process and formulation will likely be application-specific.

7. Future research

Areas for future research can be organized into three interconnected themes: analytical technologies, mechanistic understanding, and process development (Table 2).

Table 2

Overview of the future research themes and the associated knowledge gaps discussed in this Review.

Research theme	Key research areas
Mechanistic understanding	Analytical gaps; formulation dependence of population drift; membrane interactions and selection; impurity control.
Process development	Upstream–downstream integration; operating windows; scale-up; manufacturing-relevant process development.
Analytical technologies	In-line and at-line analytics; single-particle characterization; process analytical technology (PAT); monitoring of subpopulation dynamics.

7.1. Analytical technologies

A central constraint in LNP manufacturing is the analytical gap. First, even when suitable characterization techniques exist, they are typically slow, resource-intensive, technically demanding, and offline, limiting visibility into product-state evolution during downstream processing. In-line or at-line monitoring of relevant LNP attributes remains largely absent. Characterization techniques are often method or operator dependent, like those to assess biological potency, where available *in vitro* assays lack standardization.

Second, many commonly employed analytical approaches rely on ensemble-averaged surrogates that mask population heterogeneity, including in payload distribution [7,63,119,120] and morphology [7,16,25,27], thereby obscuring rare but critical subpopulations. High-resolution, single-particle techniques capable of resolving LNP, including cryo-TEM, nanoflow cytometry, and other platforms such as cylindrical illumination confocal microscopy (CICS) [63,68], convex lens-induced confinement (CLIC) [121], and single particle automated Raman trapping analysis (SPARTA) [122,123] are throughput-limited. As a result, their integration into routine PD is often still limited.

Addressing these analytical gaps by expanding the analytical toolbox in terms of throughput, speed, and information content, and by enabling in-line or at-line characterization, would unlock additional capabilities for real-time process monitoring, modeling, and mechanistic understanding of how downstream operations shape LNP quality attributes. Improved resolution of population heterogeneity would permit systematic assessment of subpopulations emergence, evolution, and transformation during downstream processing, and would clarify to what extent heterogeneities are functionally relevant and must be controlled within defined bounds during LNP manufacturing.

7.2. Mechanistic understanding

7.2.1. Formulation dependence of population drift

Multiple downstream processing steps measurably influence LNP quality attributes, most commonly through population level drift. However, there are currently no generally transferable heuristics linking downstream process conditions to the magnitude of such drift. Moreover, downstream invariance cannot be assumed when cargo identity, formulation composition, or surface modifications change, with direct implications for LNP platform claims that presuppose formulation and cargo-independent process–quality relationships.

The academic literature offers limited mechanistic insights into why nominally similar LNP formulations can exhibit disproportionate sensitivity to downstream processing, despite such effects frequently determining manufacturability. This gap likely reflects a bias toward reporting stable formulations rather than formulation-specific failure modes. Addressing this is a clear opportunity for future research: systematic investigation of formulation-dependent downstream behavior could enable formulation choices to be informed not only by upstream assembly and biological performance, but also by robustness to downstream processing during lipid screening and process design.

7.2.2. Membrane interactions and selection

Membrane-based operations dominate LNP downstream processing, yet LNP-membrane interactions remain poorly understood, leaving membrane selection based primarily on experimental screening. The influence of LNP physicochemical properties on membrane performance has been only sparsely evaluated. Consequently, membrane selection remains largely empirical, with few generalizable design rules beyond broad recommendations regarding membrane material and MWCO. Future work should elucidate how membrane surface chemistry (e.g., RC and mPES) and LNP properties such as hydrophobicity, surface charge, and roughness govern LNP adsorption, deformation, and fouling behavior, which impacts process yield, robustness, and scalability.

This gap is particularly consequential for surface-modified LNPs,

which are increasingly explored to enable extrahepatic delivery [21,22]. Conjugated targeting moieties alter the LNP interface and can exhibit (quasi-)specific interactions with the membrane. The impact of these targeting surface modifications on membrane-based separations is unresolved. At the molecular level, interactions between specific functional groups and membrane surfaces can be systematically interrogated using techniques such as atomic force microscopy [124], enabling the quantification of interaction forces relevant to membrane-based downstream processing.

7.2.3. Impurity control

LNP downstream unit operations effectively remove process-related impurities that differ considerably in size or permeability (Sections 3, 5). More problematic are product-related impurities, which cannot be removed once incorporated into the final drug product. Regulatory assessments of commercial LNP DPs recognize this distinction by evaluating not only process-related impurities but also product-related species such as truncated and modified RNA and lipid degradation products [125]. One example is described by Packer et al. [126], where reactive degradants of ionizable lipids form covalent adducts with mRNA, impairing therapeutic translation. Impurities are usually mitigated through downstream separation, but such lipid-derived impurities cannot be removed once formed. Their control therefore depends on understanding how downstream unit operations influence their generation, including solvent history, pH transitions, residence time, and oxidative exposure. From this perspective, downstream operations should not be viewed solely as purification steps, but as active parts of an impurity-prevention strategy. Future work should clarify how individual unit operations contribute to impurity formation and integrate these insights into PD.

7.3. Process development

7.3.1. Upstream-downstream integration

Improved upstream-downstream integration is essential for better mechanistic insights and for PD. Enhanced upstream control enables systematic interrogation of downstream-induced population drift under stable initial conditions, isolating effects previously confounded by upstream variability. For PD, downstream sensitivity constrains upstream parameters. Upstream and downstream must thus be co-designed: modulation of upstream-generated LNP quality attributes can precondition LNPs to tolerate downstream stresses, mitigate aggregation and filter fouling, and widen downstream operating windows. For example, buffer exchange-induced size growth can be anticipated by operating upstream to generate smaller initial particle sizes.

7.3.2. Scale-up

Scale-up presents two major challenges. Firstly, one key scale-up limitation is the poor translatability of small-scale downstream workflows to manufacturing-scale operation, particularly for buffer exchange where dialysis-derived insights are misleading for manufacturing-scale hydrodynamic regimes. This disconnect also has important translational implications, as laboratory-scale downstream workflows may generate material that is not fully representative of the final clinical product, increasing development and regulatory risk. Recently, low-holdup-volume, small-footprint TFF systems have become commercially available, enabling buffer exchange at lower volumes under flow regimes, residence times, and shear conditions that more closely resemble manufacturing operation.

A second scale-up challenge is that certain downstream failure modes only emerge at clinically relevant batch sizes and post-processing LNP concentrations, especially for membrane operations due to nonlinear effects associated with membrane area, membrane loading, and process duration. This includes the very different hydrodynamics in pleated filters used for large-scale sterile filtration compared to that in small scale capsules employing disk filters. Addressing this gap requires

explicit identification of scale-relevant parameters and the systematic evaluation of the operating windows, rather than reliance on small-scale surrogates with limited translational validity.

The lack of cost-effective experimental surrogates further complicates downstream process development, as drug-free LNPs fail to recapitulate the behavior of cargo-loaded formulations even at small scale [31]. Establishing surrogate validity for specific downstream questions would reduce experimental burden and broaden access to mechanistic studies, especially for academia.

8. Conclusions

LNP manufacturing does not end at particle formation. Following upstream production, LNPs undergo a sequence of downstream unit operations, including buffer exchange, conditioning, several filtration steps, and fill-finish, each transforming the product state such that their cumulative effects collectively define the final DP. Far from being passive, downstream processing actively reshapes LNP quality attributes and particle populations. Consequently, it is not only a quality-defining but also a translation-enabling component of LNP manufacturing, as early downstream decisions determine how representative laboratory-generated material is of the final clinical product. Yet despite affecting manufacturability, stability, and ultimately the therapeutic performance, it remains comparatively under-studied.

The first step is recognizing the importance of downstream processing in LNP workflows. Correcting the current imbalance requires systematic, scale-relevant interrogation of individual downstream unit operations and their coupling, with explicit focus on how hydrodynamic, chemical, and mechanical regimes reshape LNP quality attributes. Table 3 summarizes the expected evolution of representative LNP quality attributes throughout a typical downstream manufacturing workflow. These physicochemical changes directly determine biological performance by governing protein corona formation (e.g., surface composition and charge), biodistribution (e.g., size and morphology), cellular uptake (e.g., size and surface properties), endosomal escape (e.g., internal structure and lipid phase transitions), and protein expression (e.g., payload distribution and RNA integrity), thereby shaping immune responses, therapeutic efficacy, and safety.

The next step is to translate these qualitative relationships into quantitative, predictive ones across different formulations and manufacturing scales. To do this, academic research must move beyond small-scale surrogates toward manufacturing-relevant operating windows and explicitly account for formulation-dependent downstream evolution. In parallel, analytics capable of resolving subpopulation dynamics across unit operations are needed to link structural drift to functional outcomes. Ultimately, robust LNP process development will depend on a holistic framework in which upstream and downstream are co-designed as a single, coupled system, and platform robustness is defined by control over the product state across the entire manufacturing train. In this view, LNP manufacturing is not a sequence of discrete stages, but a coupled process of controlled product-state evolution.

CRedit authorship contribution statement

Peter Sagmeister: Writing – original draft, Conceptualization. **Cedric Devos:** Writing – original draft, Conceptualization. **Aniket Udepurkar:** Writing – original draft, Conceptualization. **Alexander Schwenger:** Writing – review & editing, Conceptualization. **Cameron Webb:** Writing – review & editing, Conceptualization. **Austin McDonald:** Writing – review & editing. **Malcolm Berry:** Writing – review & editing. **Andrew L. Zydny:** Writing – review & editing, Supervision. **Richard D. Braatz:** Writing – review & editing, Supervision. **Allan S. Myerson:** Writing – review & editing, Supervision.

Table 3

Overview of how individual manufacturing steps influence the quality attributes of a representative LNP formulation throughout a typical downstream workflow.

Quality Attribute	Formation	Buffer Exchange	Conditioning	Sterile Filtration	Fill-Finish
Particle size	Defined	Growth/Fusion	Maintained	Selective removal	Maintained
Encapsulation Efficiency	Defined	Minor losses	Maintained	Unknown	Maintained
Morphology	Defined	Altered	Maintained	Minor changes	Maintained
Payload distribution	Defined	Redistribution	Maintained	Unknown	Maintained
Surface charge	Positive	Neutralization	Maintained	Unknown	Maintained
Internal structure	Initial	Reorganized	Stabilized	Maintained	Maintained
DP composition	Initial	Transition	Excipients added	Filtered	Maintained
DP stability	Low	Improved	Optimized	Maintained	Maintained
Sterility	No	No	No	Yes	Maintained

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Declaration of competing interest

The authors declare no competing interests.

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Data availability

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

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