# Optimizing the Performance of LNP and Liposome Therapeutics with Blood-Free Recombinant Albumin

## Literature Review

Liposomes and Lipid Nanoparticles (LNPs) are a versatile delivery vehicle for a variety of therapeutic agents including small molecules, peptides, and nucleic acids. LNPs have been used in cancer treatment, vaccines, gene therapy, medical imaging, and many more applications. Until recently, one of the largest applications for LNPs has been the delivery of cancer-targeting agents, namely Doxil™ and Epaxil™, which leverage LNPs' ability to enhance solubility and delivery of the drugs.[1] More recently, LNP technology has seen a sudden rise in popularity with its use in mRNA SARS-CoV-2 vaccines in which the LNPs encapsulate mRNA coding for the SARS-CoV-2 spike protein.[1] Another notable application is gene therapy delivery (ex: CRISPR/Cas 9, siRNA, DNA) in which LNPs, typically composed of cationic or ionizable lipids, naturally complex with anionic nucleic acids and are efficient in facilitating the uptake and intracellular release of the genetic material.[2,3,4] Despite the versatility of LNPs ability to deliver genetic material and therapeutic agents, several technical hurdles still exist. These include a low circulatory half-life, lack of targeted delivery, and toxicity. The incorporation of albumin as a nanoparticle coating can be a possible solution to circumvent these pharmacokinetic challenges and potentially optimize the overall performance of these therapeutics.

Liposomes and LNPs are lipid-based particles in which the lipids, containing a hydrophilic head group and hydrophobic tails, spontaneously form a bilayer or monolayer in aqueous environments (Figure 1).[2] Liposomes are considered the predecessor of the LNP, although the term "LNP" is more widely used to refer to all classes of lipid-based nanoparticles, including liposomes. [1] LNPs have the unique ability to bind and encapsulate hydrophobic substances that typically require harsh surfactants for solubilization and can facilitate the delivery of delicate biological material that would normally be degraded in the bloodstream.[1]

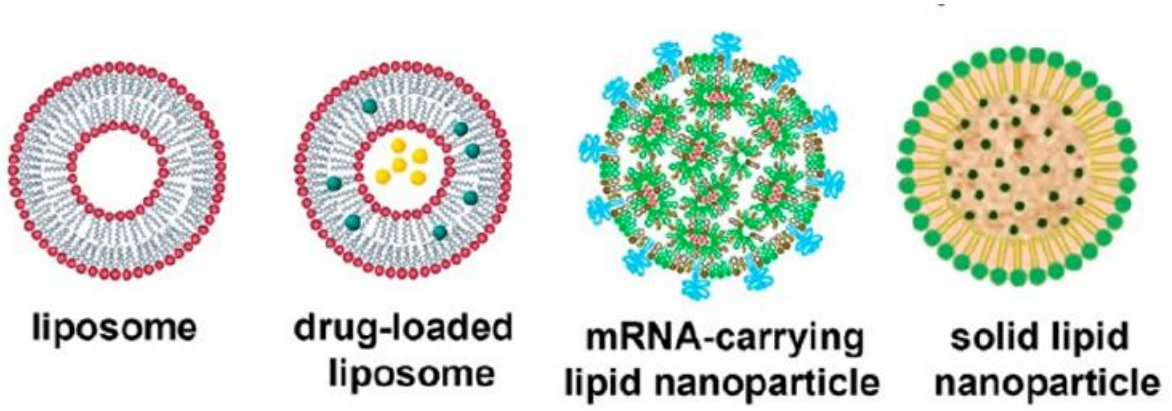


**Figure 1.** The lipid bilayer and monolayer structures of liposomes and LNPs and their ability to encapsulate therapeutic agents. This image is courtesy of Tenchov et al. 2021.[1]

One of the current challenges facing LNPs is half-life. LNPs are rapidly opsonized and subsequently phagocytosed leading to instability *in vivo* and a very short blood circulation time. While in the blood stream, nonspecific interactions with plasma proteins causes a protein corona to form on the surface of the LNPs which can change particle size and surface charge, directing the destination of the nanoparticles *in vivo*.[5,7] Finally, while synthetic lipids are effective in encapsulating and delivering therapeutic materials, these lipids can cause systemic toxicity, particularly the cationic lipids leveraged in nucleic acid delivery for vaccines and gene therapies as they can trigger apoptotic and inflammatory pathways.[3,9,13] Ionizable lipids are one strategy to combat toxicity as the lipids become neutral at physiological pH, however a tremendous amount of time and resources can be needed to develop novel lipids that can both effectively encapsulate and deliver the therapeutic while minimizing toxicity.[14,15]

Albumin presents a unique solution to address these shortcomings of LNP technology. The protein can be leveraged as a coating on the outside of LNPs to alter the pharmacokinetic properties of the nanoparticles and potentially optimize *in vivo* performance. There are 2 main strategies to do so; (A) Due to albumin's innately anionic nature, it can readily adsorb to the outside of cationic and ionizable lipids. This same mechanism is why cationic LNPs are so widely used for encapsulating anionic genetic material. (B) An albumin-polymer conjugate can also be used to covalently link the albumin to the outside of the nanoparticle (Figure 2).[6,16]

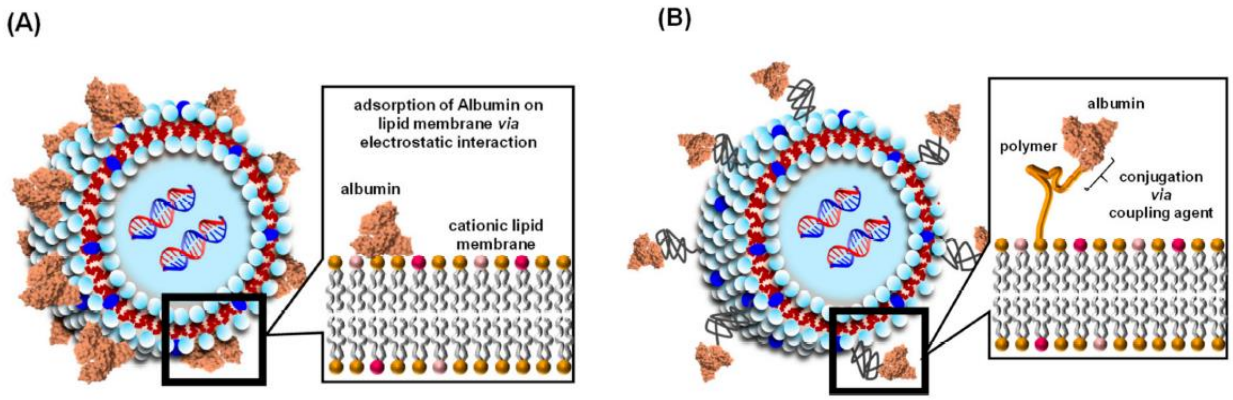


**Figure 2.** Two strategies for creating a pre-formed albumin coating on the outside of lipid-based therapeutics. This image is courtesy of Taguchi et al. 2021.[6]

This pre-formed albumin corona, whether adsorbed or covalently attached, can increase stability *in vivo*, prevent redirection by serum proteins while enhancing targeted delivery, and reduce toxic effects.[6] The albumin coating prevents opsonization and phagocytosis by creating a barrier, leveraging steric hindrance to prevent degradation form serum proteins and block detection from macrophages (Figure 3).[1, 5, 12] One study, looking at human serum albumin (HSA) coated LNPs for the delivery of siRNA to breast cancer, compared the performance of siRNA LNPs and HSA-coated siRNA LNPs to study the effect on LNP stability in the presence of serum.[3] They found that in the presence of serum, HSA-coated siRNA LNPs had a significantly greater effect on the desired genetic downregulation compared to the non-coated siRNA LNPs, most likely due to increased serum stability.[3] Because albumin is the most abundant plasma protein and is ubiquitously biocompatible, no toxic effects are realized from the albumin. Another benefit to this preformed corona is its ability to prevent redirection and cellular uptake inhibition by non-specific plasma proteins adsorbing to the LNP. [6]

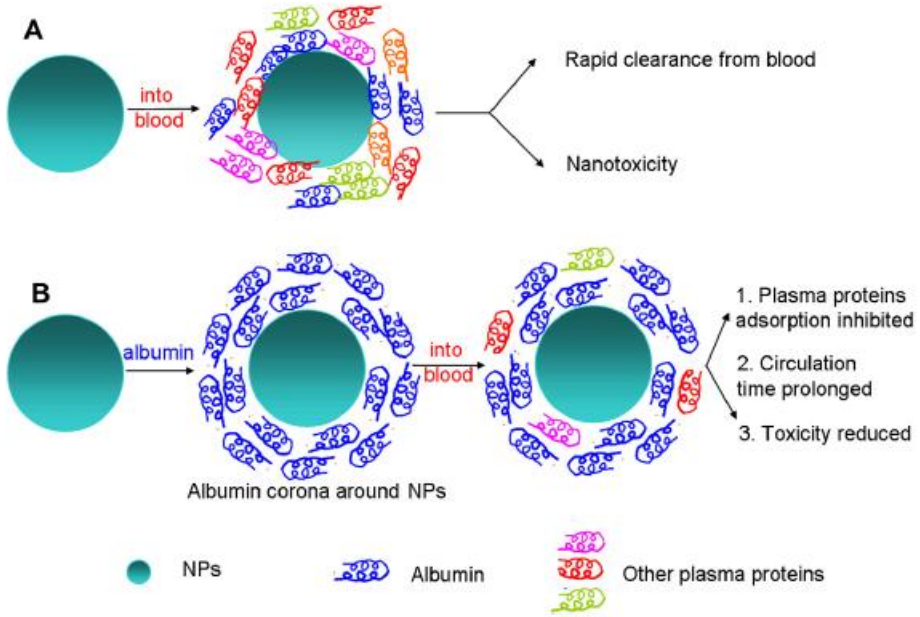


**Figure 3**. Albumin corona blocking the formation of non-specific plasma protein interactions on nanoparticle technology. This image is courtesy of Peng et al. 2013 [5]

Nucleic acid LNPs for gene therapy are an attractive option compared to the traditional vector-based delivery methods such as AAV, which can cause immunogenic reactions and are unable to be re-dosed due to clearance by the body's immune system.[4] While LNPs typically have low immunogenicity and allow for re-dosing, LNP gene therapies also have their own drawbacks including variable transfection rates. Leveraging the mechanisms described above, albumin coatings can enhance transfection efficiency by preventing inhibition from serum components and resistance to serum endonucleases.[3] Albumin-binding proteins, gp30 and gp18, can also facilitate enhanced endocytosis of albumin-associated LNPs.[16]

Another unique characteristic of albumin is its ability to enhance the targeted delivery of therapeutics to specific cell types and cancers. Certain cell and tumor types overexpress secreted protein acidic and rich in cysteine (SPARC).[12] This extracellular matrix glycoprotein has an extremely high binding affinity for albumin; thus an albumin corona can direct the LNPs to these high SPARC producing cells. The enhanced permeability and retention (EPR) effect seen at tumor and inflammatory sites can also lead to the passive accumulation of albumin and albumin associated LNPs, further enhancing targeted delivery of the associated therapeutics.[1,6,12]

While a pre-formed albumin corona has the potential to optimize the performance of LNP-based therapeutics, the use of animal component-free, recombinant albumin could further

enhance performance and ensure higher consistency and safety. Historically, serum albumin has been sourced from bovine and human blood. To remove any adventitious agents, these albumins undergo chemical inactivation which can cause protein denaturation and reduced functionality. This can further compound donor-to-donor variability which manifests in inconsistent binding capabilities of albumin from different donors, leading to extreme lot-to-lot variability with serum-derived albumin.[17] With the rise in COVID-19 pandemic related supply chain shortages, so came a serum and serum-albumin shortage that impacted both pre-clinical and clinical stage vaccine and biopharmaceutical companies, stalling many programs. These supply chain shortages continue to afflict therapeutic pipelines with seemingly no end in sight as blood and plasma donations decrease as demand skyrockets, further exasperating serum protein shortages.[18]

InVitria's recombinant human serum albumin (rHSA) is produced using a highly scalable, non-mammalian expression system. Removing donor variability and the need for heat or chemical inactivation steps, InVitria's rHSA offers extremely high lot-to-lot consistency. This, combined with supply chain continuity and risk mitigation of introducing adventitious agents, can help to create a safer, more scalable therapeutic. InVitria produces two different rHSA products suited for the use with LNP technology: Exbumin™ and Optibumin™. Exbumin is a lyophilized rHSA designed for use in the final formulation of therapeutics. Exbumin is the only rHSA approved for use as a final formulation excipient by the FDA and EMA in biologics such as Merck's ERVEBO vaccine.[8] Optibumin, provided as a 10% liquid formulation, is the highest purity albumin on the market. Optibumin is also lipid-stripped and has a high mercaptoalbumin content making it the ideal albumin for enhanced binding to lipids. The high monomer purity and lipid-stripped nature lends this albumin to potentially have an increased binding affinity for the cationic surface of LNPs, furthering the benefits seen with pre-formed albumin coronas. The free Cys-34 binding site can also be leveraged to enhance albumin-polymer conjugation for covalently forming the albumin corona. As LNP technology continues to advance and new therapeutics enter the market, the need for albumin will continue to increase. Having a reliable, recombinant source that is also safe and regulatory friendly can propel projects forward above the rest.

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