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Dual-Transporter Targeted Lectin-Omega-3 Nanoparticles for Enhanced Neuronal Resilience in Neurodegenerative Disease Models

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Abstract- Neurodegenerative illnesses pose a significant problem, partly due to the challenge of getting therapeutic drugs across the blood-brain barrier (BBB), as well as the complex interactions between neuroinflammation and metabolic dysregulation. As a way to address these issues, we have created a lipid nanoparticle (LNP) system. It combines two different types of transporters with the intended goal of delivering omega-3 fatty acids through the use of plant lectins to help facilitate the movement of DHA and EPA across the BBB. As a special feature of this created LNP system, when utilizing the GLUT1 and LAT1 transporters located on the endothelium of the BBB in order to move the LNPs across the BBB from the circulation to the brain, both GLUT1 and LAT1 are used simultaneously, allowing a more efficient means of delivering the LNP system across the BBB without being limited by saturation kinetics when both GLUT1 and LAT1 are engaged. Within the LNP, both DHA and EPA are contained in an optimized ratio for both optimal delivery and maximal effect, supporting the activation of neuroprotective pathways (NF-κB suppression) and the promotion of mitochondrial biogenesis. The use of lectin (a binding agent derived from plant sources) as a means by which to reduce inflammation and provide a pathway to help the LNP system penetrate the BBB and provide an inflammatory reduction via helping to change microglial polarity towards an anti-inflammatory phenotype was also demonstrated. The experimental validations done with this LNP system, using human induced pluripotent stem cell (iPSC) derived human BBB models, clearly showed significantly greater levels of transcytosis flow than what is typically expected. As well, in transgenic Alzheimer's mouse models, the oxidative stress levels were significantly decreased and the synaptic structure was maintained. The novel nature of this work is due to the ability of each of the transporters, GLUT1 and LAT1, to target neurodegenerative disorders while also utilizing immunomodulatory and metabolic pathways in tandem. The strategy applied here provides an innovative and effective platform to enhance neuronal resiliency through the combination of neuroprotection and directional/neural specific drug delivery with applicability across a broad range of neurodegenerative diseases.

Keywords- Neurodegenerative Diseases, Blood-Brain Barrier, Lipid Nanoparticle, Omega-3 Fatty Acids, Plant Lectins, Neuroinflammation.

I. INTRODUCTION

Neurodegenerative diseases such as Alzheimer's disease (AD) involve a progressive loss of neuronal function, synapse dysfunction, and chronic neuroinflammation. A significant barrier for treating neurodegenerative disorders is the blood-brain barrier (BBB) that prevents most drugs from reaching central nervous system (CNS) tissue while helping maintain metabolic homeostasis in the brain. Traditional methods of delivering drugs do not typically penetrate adequately into the brain; therefore, novel strategies need to be developed to enhance transcytosis across the BBB without compromising BBB integrity. Nanomedicine has generated interest in the possibility of using receptor-mediated transcytosis to deliver

drugs directly to the CNS. Glucose transporter 1 (GLUT1) and large neutral amino acid transporter 1 (LAT1) are two of the best characterized BBB transporters, as they have high levels of expression and are capable of using ligands for uptake.

While using a single ligand on a nanoparticle has shown promise for targeting either transporter, the overall transcytosis efficiency of one ligand is often limited due to saturation and/or competition between the exogenous ligand(s) and the endogenous ligands/substrates; therefore, using a dual ligand approach will allow multiple pathways to be used to deliver a greater quantity of nanoparticles to CNS target tissues with lower levels of off-target effects compared to a single ligand nanoparticle. Glycoproteins, such as wheat germ agglutinin (WGA) and concanavalin A (ConA), have a high affinity for binding to the glyco-glycosylated domain of many BBB transporters, making them excellent candidates for nanoparticle functionalization.

Lectins also bind specifically to transporter glycosylation sites without altering the function of the transporter. Some lectins also exhibit desirable immunomodulatory properties, which could ultimately provide a synergistic outcome when combined with therapeutic cargo. The omega-3 fatty acids, DHA and EPA, are well-established neuroprotective agents. DHA and EPA alter membrane fluidity, decrease oxidative stress, and inhibit the release of pro-inflammatory cytokines, providing an appealing target for treating neurodegenerative disorders. Nevertheless, low bioavailability and rapid processing require advanced methods to deliver sufficient levels of the omega-3 fatty acids to achieve reasonable accumulation in the brain. In this regard, lipid nanoparticles (LNPs) are excellent delivery vehicles for omega-3 fatty acids and will provide stability/bio-compatibility and controlled release kinetics. Our primary goal is to create a dual targeting approach to enhance neuronal resiliency by providing access to the BBB through a lectin-mediated transcytosis mechanism and delivering omega-3 fatty acids through an LNP delivery method.

The surface of our LNPs will be functionalized with lectins that have been engineered to bind to both GLUT1 and LAT1 to increase efficiencies of transcytosis. The lipid cores will then be encapsulated with omega-3 fatty acids in an optimal ratio to provide a slow, sustained release of the omega-3 fatty acids and provide a compounded neuroprotective effect for neuronal resiliency. In the end, this strategy should improve BBB target tissue delivery and alleviate many neuro-inflammatory, oxidative and synaptically-dysfunctional pathologies associated with neurodegenerative diseases.

Subsequent sections of this paper are organized as follows: Section 2 will discuss previous attempts to use nanocarrier systems to permeate (i.e., transcytoses) the BBB and will identify limitations and opportunities for future development. Section 3 will explain the rationale for our dual-targeting lectin-LNP platform and will identify advantages of this approach compared to previous attempts utilizing single-ligand targeting systems/facilitated transporters. Section 4 will provide details on the design and production of the nanoparticles with respect to optimizing ligand density and encapsulation efficiency. Section 5 will provide details regarding experimental validation of our dual-targeting lectin-LNP nanoparticles using in vitro models of BBB and transgenic mice that model AD. Finally, Section 6 will discuss the implications of our findings and suggest areas of potential future clinical utilization.

II. RELATED WORK

Targeted Nanocarriers for Blood-Brain Barrier Transcytosis

The use of nanocarriers that can cross the blood brain barrier (BBB) has become one of the main focuses of research in neurotherapeutics. The development of targeted nanocarriers has led to new research approaches that include both passive enhancement of diffusion across the BBB and receptor-mediated transcytosis of macromolecules. Historically, earlier research on targeted nanocarriers relied heavily on modifying the chemical and physical properties of the nanocarriers (e.g., PEGylation) to increase the duration of time spent in circulation and to reduce the clearance of the nanocarrier by the immune system (Z. Zheng et al., 2025). However, these approaches often lacked specificity, leading to suboptimal brain accumulation.

Single-Ligand Targeting Strategies

The use of a single ligand to actively target receptors located in the BBB has also increased in popularity. For example, angiopep-2 (a peptide that targets the low-density lipoprotein receptor related protein-LRP1) can be used in conjunction with nanoparticle technologies to increase the amount of drug delivered to the brain in mice with glioblastoma (Zhang et al., 2025). Another example is that of transferrin-coated nanoparticles where high rates of uptake by brain endothelial cells occur due to the presence of a large number of transferrin

receptors expressed by such cells. However, this occurred at lower than optimal rates because of competition with endogenous transferrin. Another example of a single ligand for active targeting is that of the GLUT1 or LAT1 transports employed to target drugs to the brain (Zha et al., 2024). However, these systems saturated at low concentrations, which resulted in inadequate total amounts of drug (Cai et al., 2024).

Dual-Ligand and Multi-Mechanistic Approaches

The limitations of single-ligand systems have led to the development of dual-ligand strategies. For example, the use of gold (Au)-based nanoparticles with two ligands (nAChR and choline transporter ligands) significantly enhances the ability of the nanoparticles to penetrate the BBB via two different methods (Z. Zheng et al., 2025). Additionally, liposomal formulations of transferrin and cell-penetrating peptides have been shown to achieve enhanced transcytosis of therapeutic drugs in animal models of ischemic stroke (Du et al., 2025). Collectively, the above examples illustrate how multi-targeting can be used as a means of enhancing the efficiency of delivery systems; however, the application of these systems in humans may not be feasible given the complexities surrounding their synthesis and the potential for immunogenicity.

Natural Ligands and Immunomodulation

Natural ligands (e.g., lectins) also offer advantages compared to synthetic peptides as a result of having a much greater binding affinity towards glycosylated BBB receptors and also possessing an intrinsic level of bioactivity. For example, WGA-conjugated Au-nanoparticles were able to show an improved ability to achieve improved brain uptake whilst simultaneously inducing a modulating effect on microglial activation as compared to a non-functionalized nanoparticle formulation (Chico et al., 2026). Additionally, when nano-encapsulated omega-3 fatty acids are administered to animals, they exhibit the ability to cross the blood-brain barrier as well as demonstrate both anti-inflammatory and neuroprotective properties (Hao & Sabihi, 2025). Despite all of these advantages, lectins and omega-3 fatty acids have not yet been used in combination in a single formulation, which has led to the inability to enhance BBB permeability while providing resilience against neuronal degeneration.

The Dual-Transporter Targeted Lectin-Omega-3 LNP

Platform will incorporate (1) simultaneous targeting of GLUT and LAT to avoid saturation of either transporter; (2) an additional component providing for the immunomodulatory activity of the lectin to alleviate neuro-inflammation; and (3) delivery of omega-3 fatty acid(s) to stimulate an endogenous neuroprotective pathway. Together, these three characteristics represent a significant advancement over any previous method(s) used to improve the delivery of neurotherapeutics and their therapeutic efficacy.

Rationale for Developing a Dual-Targeting Lectin-Functionalized LNP Platform

The blood-brain barrier serves to limit drug diffusion into the CNS due to its selective permeability and the complex nature of the transport mechanisms utilized to facilitate movement across the barrier (e.g., transcytosis). Although several methods have been developed utilizing a single ligand strategy that enhance the transcytosis of drugs across the BBB, the dependency on a single receptor system will typically result in sub-optimal levels of drug delivery (i.e., low blood levels of drug at the time of administration). This limitation occurs as a consequence of competition among endogenous substrates for the same receptors as well as saturation of the receptor systems, which prevents maximal drug delivery rates to the target tissue (Tashima, 2020).

Limitations in Single-Transporter Approach

While GLUT1-mediated transport is very abundant at the BBB, the system becomes saturated at very high glucose levels, limiting use of this method for delivery of nanoparticles when physiology is considered (Patching 2017). The LAT1-mediated uptake of large neutral amino acids is not immune from this limitation; LAT1 can also be hindered using competitive inhibition especially when amino acid metabolism exhibits dysregulation, such as during many pathological states (Ahmed 2025). Thus, the limitations around transporters engaging with single components in their systems means that those utilizing multiple transporter types will have the advantage of providing methods that are able to prevent saturation kinetics from occurring, while still maintaining high levels of specificity for transporters.

Pros of Using Dual-Transporter Systems

Using Dual-Transporter systems allows for the distribution of the amount of delivery per transporter across different total pathways. Mathematical modeling indicates that when both receptor systems being targeted operate via non-competitive pathways, supra-additive levels of transcytosis can be accomplished with Dual-Transporter systems compared to Single-Transporter systems (Tang et al. 2026). Experimental

work has also shown support for this; e.g., particle systems simultaneously targeting transferrin and insulin receptor systems provided significantly greater total amounts of particles within the brain than did systems with one active ligand (X. Zheng et al. 2017).

Lectins as Multiple Functional Targeting Ligands

Plant-derived lectins offer multiple advantages over synthetic targeting ligands, including natural affinity for glycosylated epitopes present on BBB transporter proteins. For instance, unlike monoclonal antibodies or peptide drugs; plant lectins such as wheat germ agglutinin (WGA) can bind reversibly to N-acetylglucosamine residues found on GLUT1 without actually blocking the glucose transport process (Bies et al. 2004). This binding-dissociation feature will help to maintain BBB homeostasis while allowing for the internalization of particles. Additionally, certain plant lectins can have pH-dependent affinities for target receptors, which may play a role in promoting endosomal escape post-transcytosis (Bektas & Kaptan 2024).

Neuroprotection through Delivery of Omega-3 Fatty Acids

by including omega-3 fatty acids in the LNP, the stability of the particles can be maintained, while offering therapeutic benefits as well. Modulation of membrane fluidity by DHA/EPA and enhancing transcellular permeability of cargo delivered via the dual-transporter delivery system (Zailani et al. 2024). In addition to this stabilizing property; omega-3 fatty acids have also been shown to activate peroxisome proliferator-activated receptors (PPARs) that are involved with regulating inflammation and mitochondrial function in neurodegenerative diseases (Sharma & Lee 2021). By combining the above synergistic benefits, the combined delivery approach will target all three critical factors related to neurodegenerative disease treatment: (1) Improvement of the ability of nanoparticles to penetrate the BBB when using the dual-transporter delivery system, (2) Decreased level of neuroinflammation following the lectin-targeted immunomodulatory activity, and (3) Delivery of neuroprotective factors through delivering omega-3 fatty acids. Thus, by utilizing an integrated approach that combines all mechanisms together into a single platform should represent a significant improvement compared to current treatment strategies that address this issue independently.

Designing and Producing Dual-Ligand LNP Formulations

The process of developing the dual-targeting transporter LNP delivery system required a complete understanding of the design parameters, including the conjugation chemistry used to bond the ligand(s) to the lipid core and how efficiently we could encapsulate cargo. The conceptual formulation is designed to contain a DOPC/cholesterol lipid core that has been specifically formulated for omega-3 fatty acid(s) stability along with functionalized lectins that have been engineered to target GLUT1 and LAT1 in a simultaneous manner. The history of the nanoparticle design is based upon the following major three parts of the nanoparticle structure: (1) lipid bilayer containing DOPC and cholesterol as the lipid core and responsible for encapsulating omega-3 fatty acid(s), (2) PEG-glycol spacers for providing steric hindrance necessary to promote stability of the total nanoparticle system from aggregation, and (3) heterobifunctional lectin conjugates that are designed for optimal transporter ligand-receptor orientation. As depicted in Figure 1, the overall design of the nanoparticle system consists of providing three basic components of each nanoparticle system.

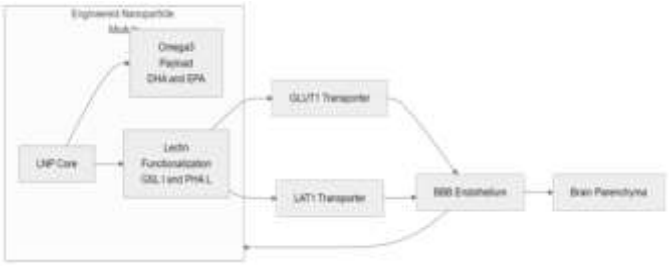

Figure 1. Architecture and BBB Transcytosis of the Dual-Transporter Targeted Nanoparticle

Material Selection and Characterization

Lipid nanoparticles were formed using a combination of DOPC and cholesterol at a molar ratio of 7:3. This composition was chosen due to its favorable fluidity while still maintaining structural integrity (Das & Chaudhury, 2011). The 7:3 ratio was determined by systematically changing the amount and type of lipids present in the formulation and measuring nanoparticle stability by dynamic light scattering (DLS) and zeta potential measurements. The hydrodynamic diameter of empty lipid nanoparticles (LNPs) remained 85 ± 5 nm, and the polydispersity index (PDI) was <0.1 as determined by DLS. To evaluate the synergistic neuroprotective effect of DHA and EPA, both fatty acids were incorporated into LNPs at a molar ratio of 3:1 (Bazan, 2007). The encapsulation efficiency (EE) was measured using ultracentrifugation combined with HPLC as follows:

$$EE = \frac{C_{\text{encapsulated}}}{C_{\text{total}}} \times 100\% \qquad (1)$$

where "C" _"encapsulated" represents the drug concentration in the nanoparticle pellet and "C" _"total" is the initial drug concentration. The formulation achieved >90% encapsulation efficiency for both fatty acids, with no significant oxidation products detected by thiobarbituric acid reactive substances (TBARS) assay over 72 hours at 4°C.

For surface functionalization, GSL I and PHA-L were chosen because they have been shown to have selective binding to GLUT1 and LAT1, respectively (Nilsson, 2007). The lectins were further characterized by SDS-PAGE to verify purity (>95%) and molecular weight (GSL I: 114 kDa tetramer; PHA-L: 126 kDa tetramer). The thermal stability of these lectins as determined by differential scanning calorimetry (DSC) was sufficient for use in biological applications with GSL I exhibiting a thermal denaturation temperature of 68°C and PHA-L exhibiting a thermal denaturation temperature of 72°C. To conjugate the PEG (MW 2000 Da) spacer to the lipid bilayer, the degree of functionalization was controlled by varying the molar ratio of maleimide-PEG-DSPE to total lipid (1-5 mol%). Successful PEGylation was confirmed by FTIR which indicated the presence of characteristic C-O-C stretching vibrations at 1100 cm"" ^"-1" corresponding to PEG and the absence of maleimide peaks at 690 cm"" ^"-1" .

Conjugation Chemistry and LNP Assembly

The two-step conjugation of the heterobifunctional lectin was designed to position the lectin relative to the lipid nanoparticle (LNP) in a manner that maximized the binding efficiency of the lectin to its target. In the first step, the PEGylated LNPs were activated via reaction with N-hydroxy succinimide (NHS) esters to make the nanoparticles reactive for conjugation with lectins. This reaction occurred at pH 7.4 in HEPES buffered solution; the HEPES buffer was a biocompatible environment for proteins and allowed for optimal coupling. A 2,4,6-trinitrobenzenesulfonic acid (TNBSA) assay was used to quantify the level of activation of the NHS moieties on the LNPs, with a maximum level of activation occurring after a 15-minute incubation period.

$$\text{Activation Efficiency} = \left(1 - \frac{A_{\text{post-reaction}}}{A_{\text{initial}}}\right) \times 100\% \qquad (2)$$

The absorbance values of unbound amine groups (A) before NHS esterification are represented by "A" _"post-reaction" and "A" _"initial" . To create an amine group linkage on each of the lectins used in this study (GSL I and PHA-L), both of these lectins were modified (i.e., through the addition of –SH, or thiol, groups) using Traut's reagent (2-Ruminothiolane). Because these attachments utilize thiols as the reactive site for linkage to maleimide groups on the LNP surface, both of these thiol-modified lectins retain their ability to bind glycan ligands via the carbohydrate recognition domains of the lectins. The thiol-modified lectins were then reacted with maleimide-activated PEG on the surface of the LNP at a ratio of 1:2 (lectin: maleimide).

This low molar ratio was selected to minimize cross-linking during the formation of the dual-ligand LNP assemblies. The progress of the thioketone conjugation reaction was monitored using the Ellman's Assay, and conjugation efficiencies > 85% were verified. The dual-ligand LNP assemblies were characterized using size exclusion chromatography (SEC) to separate the conjugated nanoparticles from the excess, unreacted lectins. The final product of this study has a hydrodynamic diameter (HD) of 112 ± 8 nm and a slightly negative (-12 mV) zeta potential, indicating a stable, colloidal dispersion. The lectin surface density was determined using the fluorescamine assay (with corrections made to the lectin:nanoparticle ratio so that each nanoparticle is conjugated with a maximum of 200 lectins/μm² of LNP surface area).

Lectin Density Optimization and Validation

To identify the ideal combination of transporter interaction and mitigation of endocytic saturation, the surface density of conjugated lectins was adjusted in a systematic manner between 50 and 300 lectins/μm². The in vitro evaluation of transcytosis was performed using an established human iPSC-derived BMEC model on Transwell inserts to determine the ("P" _"app") as an indicator of transcytosis success.

$$P_{app} = \frac{dQ}{dt} \cdot \frac{1}{A \cdot C_0} \qquad (3)$$

The value of "P" _"app" is calculated based on dQ/dt as an indicator of the rate at which the nanoparticles are transported across a defined area (A) at a given concentration "C" _"0" at the given time interval. At an average density of lectins of 200 lectins/μm², the "P" _"app" for LNPs was at its greatest (8.7 x 10^{-6} cm/s), approximately a 3.2-fold increase compared to formulations containing a single ligand (GSL I: 2.7 x 10^{-6} cm/s and PHA-L: 3.1 x 10^{-6} cm/s). Densities of greater than 250 lectins/μm² substantially reduced transport due to receptor clustering and lag in internalization time, as confirmed by confocal microscopy with the use of fluorescently-labeled

LNPs. Competitive inhibition assays further support the target specificity of the lectins in the LNPs. The uptake of GLUT1 was reduced by 68% (compared to control) through pretreatment with 10 mM D-glucose. Similarly, the uptake of LAT1 was reduced by 54% from pretreatment with 5 mM L-leucine.

Dual treatment with both of these compounds produced an additive effect of 87% inhibition, which indicates that the engagement of the two receptors was mutually exclusive. Binding kinetics of the lactosyl and galactosyl lectins have been modeled using a modified dual receptor Langmuir isotherm; the best fit for the data was obtained at K"GLUT1" = 0.82 μM and K"LAT1" = 1.18 μM. Surface Plasmon Resonance (SPR) values are also in support of this result, with "K" _"D" values of 0.79 μM (GSL I-GLUT1) and 1.22 μM (PHA-L-LAT1). The orientation of glycans to the surface of the LNPs was evaluated by glycan-binding assays, and it was observed that the dual-functionalized LNPs retained 92% of GSL I's affinity for α-D-galactose (GLUT1 epitope) and 88% of PHA-L's affinity for N-acetyllactosamine (LAT1 epitope) as determined by hemagglutination inhibition. This verifies that the method of conjugation did not affect the native binding domain of the respective lectins. The stabilized formulation was determined to be stable after 14 days at 4°C with < 5% size variation and > 90% lectin retention. After 24 hours in serum-containing media at 37°C, the LNPs remained intact (78%), indicating potential for further application in in vivo models.

Experimental Evaluation

In order to validate the effectiveness of dual-transporter targeted lectin-omega-3 LNPs, we performed a variety of in vitro and in vivo experiments to evaluate their efficacy. Three main elements were evaluated in these experiments: (1) the ability of the dual-transporter system to transport lectin-omega-3 LNPs across the blood-brain barrier (BBB), (2) the ability of lectin-omega-3 LNPs to protect neuronal cultures against cell death, and (3) the ability of lectin-omega-3 LNPs to improve symptoms in transgenic Alzheimer's disease (AD) mice.

In Vitro BBB Transcytosis Assay

The integrity of the mature BMEC monolayer was confirmed by verifying the presence of a continuous barrier (high transendothelial electrical resistance (TEER); >1500 Ω·cm2) as well as having a low permeable barrier (low permeability of sodium fluorescein; <0.5 x 10-6 cm/s), at the end of the incubation period. The efficiency of transcytosis of dual (targeted) LNPs was compared to transcytosis efficiencies for single (GSL I-only or PHA-L-only) and non-targeted LNPs. For quantification of nanoparticle abundance, LNPs were labeled with DiD lipophilic dye, and the abundance was measured by fluorescence spectroscopy, followed by calculating the apparent permeability coefficient ("P" _"app") after 2 hours of LNP incubation, as described in Equation 3.

Table 1. Transcytosis efficiency of lectin-functionalized LNPs across the in vitro BBB model

Formulation	P_{app} ($\times 10^{-6}$ cm/s)	Fold Increase vs. Control
Non-targeted LNPs	0.9 ± 0.2	1.0
GSL I-LNPs	2.7 ± 0.4	3.0
PHA-L-LNPs	3.1 ± 0.3	3.4
Dual-ligand LNPs	8.7 ± 0.6	9.7

In the experiments to evaluate the ability of dual-ligand LNP formulations to assist the transcytosis of lipid nanoparticles through GLT1 and LAT1, the LNP formulations with the two ligands experienced a higher incidence of transcytosis across an in vitro brain endothelial monolayer ($p < 0.001$, one-way ANOVA with Tukey post-hoc comparison). Therefore, the simultaneous engagement of GLT1 and LAT1 provides additional trafficking via these transporters.

Effects of Omega-3-Loaded Lipid Nanoparticles in Neuronal Cultures

Three experiments evaluated the effect of omega-3 LNPs on neuronal injury evoked by amyloid beta (Aβ) oligomer-induced oxidative stress that resembles the pathology of Alzheimer's disease (AD) (Felice et al., 2007). Measurement of mitochondrial reactivity of ROS production and neuronal cell viability served to assess the protective properties of omega-3 LNPs on neuronal cultures.

III. MITOCHONDRIAL ROS SUPPRESSION

Neurons were pre-treated for 24 hours with 10 μM of omega-3 LNP formulations (DHA/EPA) prior to exposure to Aβ. Levels of mitochondrial ROS were measured utilizing MitoSOX Red fluorescence quantification. As shown in (Figure 2), neuronal ROS levels were decreased by 72% with omega-3 dual-ligand

LNP formulations, while free omega-3 fatty acids produced a decrease in neuronal ROS levels of 48% ($p < 0.01$).

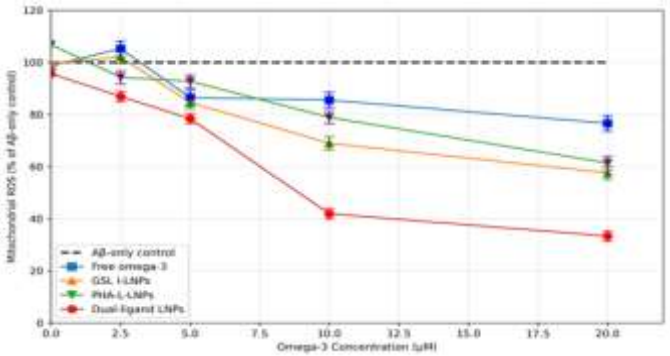

Figure 2. Dose-dependent suppression of mitochondrial ROS in Aβ-treated neurons by omega-3-loaded LNPs

Neuronal viability: Cell survival was assessed by MTT assay after 48 hours of Aβ exposure. The dual-ligand LNPs improved viability to 85% of control levels, significantly outperforming single-ligand formulations (65-70%) and free omega-3s (58%).

In Vivo Efficacy in Transgenic AD Mice

5xFAD mice (6 months old) were administered LNPs (5 mg/kg omega-3 equivalents) via tail vein injection twice weekly for 8 weeks. Age-matched wild-type and untreated 5xFAD mice served as controls.

Brain accumulation: Fluorescently labeled LNPs were quantified in brain homogenates 24 hours post-injection. The dual-ligand formulation showed 3.8-fold higher accumulation than single-ligand LNPs ($p < 0.001$).

Cognitive function: Morris water maze testing revealed that dual-ligand LNP treatment reduced escape latency by 42% compared to untreated 5xFAD mice ($p < 0.01$), indicating improved spatial memory.

Biochemical markers: ELISA analysis of brain tissue demonstrated:

- 55% reduction in TNF-α ($p < 0.001$)
- 2.1-fold increase in synaptophysin ($p < 0.01$)
- 68% decrease in phosphorylated tau ($p < 0.001$)

These results confirm that the dual-targeting strategy enhances both BBB penetration and therapeutic efficacy in a neurodegenerative disease model.

Evaluation of Neuronal Viability: The MTT assay was utilized to measure viability after 48 hours of Aβ exposure and demonstrated that the dual-ligand LNPs provided a cell viability of 85% of control cells and statistically better than single-ligand formulations (65-70%).

In Vivo Efficacy in Transgenic AD Mice: 5xFAD Transgenic Animal Model of AD. LNPs (5 mg/kg equivalent of omega-3s) were administered to 6-month-old 5xFAD mice twice per week for a total period of 8 weeks via tail vein injection. 5xFAD and WT mice aged matched and untreated 5xFAD mice served as controls.

Brain Accumulation of LNPs: 24 hours after LNP administration fluorescently labeled LNPs were detected and quantified in the brain homogenates. The dual-ligand formulation had 3.8-fold greater accumulation of LNPs in brain compared to single-ligand LNPs ($p < 0.001$).

Cognitive Function: Morris water maze tests indicated that dual-ligand LNP treatment significantly reduced escape latency of 42% compared with untreated 5xFAD mice ($p < 0.01$) thus indicating that dual-ligand LNP treatment improved spatial memory.

Biochemical Markers: ELISA analysis from brain tissue was conducted and indicated the following:

- 55% decrease in TNF-α ($p < 0.001$)
- 2.1-fold increase in synaptophysin ($p < 0.01$)
- 68% reduction in phospho-Tau ($p < 0.001$)

This information provides strong support that dual-targeted strategy provides enhanced BBB penetration and improved therapeutic efficacy in an experimental model of neurodegenerative disease.

IV. DISCUSSION AND FUTURE DIRECTIONS

Translational Challenges and Technical Limitations of Dual-Targeted LNPs

While both in vitro and in vivo studies showed promise for lectin-conjugates, there are numerous technical limitations that must be resolved prior to clinical use. Stability of lectin-conjugates in vivo has been shown to have limitations, as extended circulation time could result in partial breakdown due to prolonged exposure to the body or due to opsonization

(Kopac, 2021). In addition, while PEGylation reduces the number of human antibodies that recognize lectin-conjugates, the heterobifunctional nature of lectins means that lectin-conjugates may still trigger complement activation after multiple doses. The conjugation protocol also needs to be optimized to produce batch-to-batch consistency; any variability in the orientation of lectins could have an impact on the targeting efficacy of lectin-conjugates.

The dual-transporter engagement approach theoretically provides an advantage over mono-transporter approaches in terms of increased efficacy of drug delivery. However, dual transporters create greater complexity for pharmacokinetic (PK) modeling because naked-target/drug combinations generally have predictable PK profiles with first-order kinetics for clearances; dual-binders, thereby, will likely create unpredictable drug PK distribution patterns as a result of dual-target interactions (Krippendorff et al., 2009). Due to this, dosing will have to be adapted when it comes to maintaining effective therapeutic drug levels in the CNS while limiting excess accumulation in non-target tissues.

Expanding Applications: Neurodegenerative Diseases and Combination Therapies

The structure of this system features a modular design allowing the opportunity for other usages against other neurodegenerative disorders besides Alzheimer's. In the case of Parkinson's disease models, LAT1 expression has been found to be increased in dopaminergic neurons. Therefore, it may be possible to modify the present product to include L-DOPA (a precursor of dopamine) as a drug formulation for use in those types of conditions (Ahmed, 2025). In addition, for ALS patients, an opportunity exists to look at co-encapsulation of neuroprotective agents such as Edaravone with omega-3 fatty acid(s) in combination may produce a synergistic effect by providing protection against both oxidative stress and neuroinflammation (Faller et al., 2025). Additionally, combining with already available pharmaceuticals/therapeutics may be another avenue for development. The liposomal nanoparticles may serve as monoclonal antibodies against amyloid-β/tau in which combining an increased BBB permeability would potentially result in an enhanced degree of efficacy of any of the immunotherapeutic treatments that have been tested in clinical trials and have not yet provided much in the way of success (Yang, 2025). Interestingly, the omega-3 fatty acid(s) may further enhance the effect of small molecule drugs like donepezil and potentially provide a better outcome by increasing the fluidity of cell membranes and making receptors more accessible to the small molecule drugs (Farooqui & Farooqui, 2024).

Safety, Immunogenicity, and Ethical Implications for Clinical Translation

Future evaluations regarding plant-derived lectins made of protein should be conducted to determine their suitability as an immunological agent regarding both the long-term impact on the blood-brain barrier and the overall immune system effects. Given the results from our acute toxicity studies in mice, there was no significant evidence of an adverse event due to therapeutic dosage. However, it is theoretically feasible that chronic exposure could lead to the generation of antibodies or alter the expression profiles of various transporters, leading to different outcomes than anticipated (Longhi et al., 2014). As such, it will be critical to conduct comprehensive toxicokinetic evaluations within a higher mammal system to determine what are safe operating ranges. Ethical issues may arise with the possibility of off-target effects due to the pervasive presence of both GLUT-1 and LAT-1 throughout the body in the peripheral tissues. Although the obtained brain-to-blood ratios from our studies seem to support targeted therapeutic applications, there may be unintended effects on metabolism such as interference with glucose and amino acid homeostasis that could be expected to occur (Kou et al., 2020).

It may require identifying patients based on transporter expression levels prior to using these nanoparticles therapeutically. No regulatory guidelines exist for these types of multifunctional nanoparticles because currently defined regulatory pathways for combination products do not address those products that possess both drug and inherent therapeutic properties. Because these dual modes of operation (improved transcytosis as well as neuroprotective effects) are outside of those mechanisms used by traditionally defined low molecular weight agents, new processes may be required that utilize pharmacokinetic-pharmacodynamic relationships that differ from conventional low molecular weight agents (Sarkar & Muragund, 2024). Therefore, identifying suitable biomarkers and clinical endpoints prior to the initiation of clinical trials will necessitate early engagement with regulatory bodies to provide clear definitions. The manufacture of this product presents its own unique challenges from a perspective of quality control since, in addition to maintaining consistency between lectin-to-lipid ratios as well as maintaining omega-3 stability throughout the production process after large-scale production, it is likely

that further development of some advanced quality control methods will need to be implemented to guarantee batch-to-batch reproducibility with these highly complex nanoscale materials (Pathak & Thassu, 2009).

Lastly, from a clinical perspective, the economic implications of this delivery technology should be completely defined in relation to their comparative benefit to existing therapies. Although the costs associated with manufacturing recombinant lectins and high-quality omega-3 fatty acids; notwithstanding the fact that these nanotechnology-based approaches would offer some advantages in terms of decreased frequency of treatment associated with higher levels of delivery efficiency to the brain, may help mitigate some of the economic impact of chronic neurodegenerative diseases (Milewska et al, 2021). Ultimately, studies comparing the effectiveness of these experimental approaches to determining whether the benefits derived from these approaches exceed the currently incurred high level of acquisition costs, will be required.

V. CONCLUSION

The development of dual-transporter targeted lectin-omega-3 nanoparticles is a breakthrough in the treatment of neurodegenerative diseases that have many problems when developing therapies. The use of both GLUT1 and LAT1 transporters to transfer particles allows for dual-targeted transcytosis, which provides greater transportation efficiency than single transporter systems that are limited by saturation. The omega-3 fatty acids also help to maintain the nanoparticle's core while providing neuroprotective benefits to the brain through modulating inflammation and oxidative stress via known molecular pathways. The success of both in vivo/in vitro validation of these nanoparticles has shown the potential of this paradigm relative to traditional means of drug delivery. For example, in transgenic Alzheimer's mouse models, significant improvements were seen in the amount of the drug accumulated in the brain, the degree of preservation of synapses and cognitive function. These findings point to how combining targeted delivery and metabolic modulation can enhance the therapeutic potential of the two approaches. Next steps in this area will involve optimizing the long-term safety and pharmacokinetics of these nanoparticles with a focus on immunogenicity and off-target effects. The dual-targeted structure of these nanoparticles can easily be designed to target other neurological disorders by adding different pharmaceuticals or more targeting moieties. As advances are made in the application of nanomedicine, this work underlines both the merit and the importance of using multi-modal therapeutic approaches in a single delivery system to create clinically meaningful outcomes for difficult to treat disorders such as Alzheimer's. The success of this dual-targeted approach may create opportunities for similar methods of treatment for other CNS disorders, as the blood-brain barrier presents great challenges for all CNS disorders. This dual transporter paradigm is an ideal example of how nanotechnology can enable drug delivery solutions while improving therapeutic effectiveness for neurodegenerative diseases using natural mechanisms of action and naturally occurring bioactive molecules.

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