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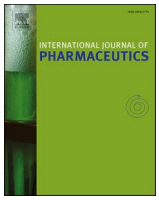

The piper at the gates of brain: A systematic review of surface modification strategies on lipid nanoparticles to overcome the Blood-Brain-Barrier

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ABSTRACT

The Blood-Brain Barrier (BBB) significantly impedes drug delivery to the central nervous system. Nanotechnology, especially surface-functionalized lipid nanoparticles, offers innovative approaches to overcome this barrier. However, choosing an effective functionalization strategy is challenging due to the lack of detailed comparative analysis in current literature. Our systematic review examined various functionalization strategies and their impact on BBB permeability from 2041 identified articles, of which 80 were included for data extraction. Peptides were the most common modification (18) followed by mixed strategies (12) proteins (9), antibodies (7), and other strategies (8). Interestingly, 26 studies showed BBB penetration with unmodified or modified nanoparticles using commonly applied strategies such as PEGylation or surfactant addition. Statistical analysis across 42 studies showed correlation between higher *in vivo* permeation improvements and nanoparticle type, size, and functionalization category. The highest ratios were found for nanostructured lipid carriers or biomimetic systems, in studies with particle sizes under 150 nm, and in those applying mixed functionalization strategies. The interstudy heterogeneity we observed highlights the importance of adopting standardized evaluation protocols to enhance comparability. Our systematic review aims to provide a comparative insight and

Abbreviations: 8314 Mab, 83-14 monoclonal antibody; Ab, Antibodies; AEGFR, Anti-epithelial growth factor receptor; API, Active pharmaceutical ingredient (API); ApoE, ApolipoproteinE.; Apr, Anti-aprotinin; AUC, Area Under the curve; BBB, Blood-brain barrier; Bend.3, Brain endothelial cells derived from mice; BMECs, Brain microvascular endothelial cells; CART, Classification and regression tree; CCR5, RNA aptamer specific for the HIV-1 entry coreceptor C-C chemokine receptor type 5; Cntn2, Contactin-2; CNS, Central nervous system; CURC, Curcumin; DHA, Docosahexaenoic acid; DHDP, 1,2-distearoyl-*sn*-glycero-3-phosphocholine, dihexadecyl phosphate; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; Dox, Doxorubicin; DSI, Drug selectivity index; DSPE, 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine; DTI, Drug Targeting Index; DTX, Docetaxel; ECs, Brain microvascular endothelial cells.; EGCG, Epigalloce-techin gallate; ETP, Etoposide; GAHBA, S-(−)-γ-amino-α-hydroxybutyric acid; GP160, RNA aptamer specific for the HIV-1 envelope protein; HA, Human astrocytes; HBVP, Human brain vascular pericytes; hCMEC/D3, Human cerebral endothelial cells; HLB, hydrophilic lipophilic balance; hiPSC, Induced pluripotent stem cell; HIV, Human immunodeficiency virus; IVIVC, *in vivo* in/*in vitro* correlation; LAT1, Large amino acid transporter 1; LDLR, low density lipoprotein receptor; Lf, Lactoferrin; LNPs, Lipid nanoparticles; MA, Anti-Melanotransferrin; MAN, Mannose; ME, Methylprednisolone; mRNA, Messenger ribonucleic acid; Nfasc, Neurofascin; NMD, Nimodipine; NGF, nerve growth factor; NLCs, Nanostructured lipid carriers; NPs, Nanoparticles; OoC, Organ-On-a-Chip systems; PAMPA, parallel artificial membrane permeability assay; Pen, Penetratin; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PCA, Principal Component Analysis; PGP, N-Acetyl-Pro–Gly–Pro; QbD, Quality by Design; QU, Quercetin; RDP, Rabies virus derived peptide; ROA, Rosmarinic acid; RVG, rabies virus glycoprotein; siRNA, small interfering ribonucleic acid; SLNs, Solid lipid nanoparticles; TA, Tamoxifen; T1/2, half life in plasma; Tf, Transferin targeting peptide; TJ, Tight junctions; Tmax, time to peak measurement; TPGS, D-α-Tocopherol polyethylene glycol 1000 succinate; TPP, Triphenylphosphine cation; VGF, nerve growth factor.; WGA, Wheat germ agglutinin.

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identify future research directions in the development of more effective lipid nanoparticle systems for drug delivery to the brain to help improve the treatment of neurological and psychiatric disorders and brain tumours.

1. Introduction

The delivery of therapeutic agents into the central nervous system (CNS) remains one of the major challenges in neuropharmacology, primarily due to the Blood-Brain Barrier (BBB) (Wu et al., 2023; Guillama Barroso et al., 2020; Tosi et al., 2019; Ding et al., 2020). Its limited permeability is essential for maintaining homeostasis, both by excluding toxins and pathogens (Akhtar et al., 2021; Bors and Erdö, 2019) and by allowing the transport of nutrients and oxygen (Wanat, 2020). However, the same permeability characteristics significantly impedes the development of treatments by excluding over 98 % of small molecule drugs and almost all large-molecule biopharmaceuticals (Pandit et al., 2020).

The BBB separates the CNS from the bloodstream and owes its permeability selectiveness to its unique vascular structure. This structure is composed of multiple cell types that together form the neurovascular unit (Wanat, 2020; Figueroa et al., 2022). The primary components of the BBB are brain microvascular endothelial cells (ECs), which differ in function and morphology from endothelial cells in other vascular systems. These cells are characterized by an absence of fenestrations which reduces surface area, and a negative surface charge due to luminal/abluminal polarization which repels polar substances (Wanat, 2020; Alahmari, 2021; Zhao et al., 2022). Moreover, they show restricted pinocytosis activity (Tosi et al., 2019; Ding et al., 2020) and yet exhibit a higher metabolic rate than other endothelial cells. This high metabolic activity is essential for the protein-mediated selective inflow of nutrients, and the outflow of specific substrates or foreign substances (Wu et al., 2023; Bors and Erdö, 2019). ECs in the BBB are closely connected due to the presence of tight junctions (TJ) and adherence junction proteins, restringing the paracellular passage of substances (Wanat, 2020; Pandit et al., 2020). Additionally, astrocytes and pericytes are two cell types surrounding the blood vessels, acting as an interface between neurons and ECs. These cell types play crucial roles in the maturation and maintenance of the BBB. For instance, astrocytes and pericytes are essential to the expression of TJ proteins and regulate the expression and localization of specific transport mechanisms across the BBB (Alahmari, 2021; Zhao et al., 2022). Astrocytes and pericytes also play a key role in regulating blood flow and vascular function, thereby modulating neuroimmune responses and waste elimination (Wu et al., 2023).

The neurovascular unit is complemented by non-cellular components such as the basement membrane and scaffolding proteins. This elements are crucial for the transporter polarization observed in the BBB and to ensure cellular stability (Pandit et al., 2020; Zhao et al., 2022). Compounds that cross the BBB must face an additional line of defense: chemical decomposition by the enzymatic activity of degrading enzymes (Wu et al., 2023; Guillama Barroso et al., 2020; Wanat, 2020). Understanding the intricate anatomical composition of the BBB and its impact on its formidable selectiveness –which favours nutrients over foreign substances– is crucial for developing effective drug delivery systems that address the clinical challenges in treating CNS diseases (Mishra et al., 2023).

Several strategies have been proposed for transporting substances across the BBB. In practice, these can be categorized into: i) non-invasive methods (both passive and active targeting) and ii) invasive methods (such as chemical or physical disruption of the BBB, or direct administration into the CNS) (Mishra et al., 2023; Khatoon et al., 2020,; Ferraris et al., 2020). However, passive targeting is typically limited to substances with low molecular weight (Nowak et al., 2020), and invasive methods are frequently associated with higher risks and potential severe side effects (Mishra et al., 2023; Upadhyay, 2014), considered as their major drawbacks. Consequently, active targeting has emerged as a promising and widely adopted strategy for overcoming the BBB. This approach utilizes targeting ligands that specifically bind to molecules exclusively present or overexpressed in the BBB (Teixeira et al., 2023). It enhances brain delivery and drug uptake by taking advantage of the high metabolic demand of the CNS and the intrinsic transports mechanisms involved in the brain's native trafficking dynamics (Mishra et al., 2023; Sánchez-Navarro et al., 2017).

In this context, the use of nanoparticles (NPs), and particularly lipid nanoparticles (LNPs), has significantly increased over the past decade, establishing a powerful strategy to enhance brain delivery of therapeutics (Tosi et al., 2019; Ding et al., 2020; Ferraris et al., 2020; Nowak et al., 2020; Ekhator et al., 2023; Kadari et al., 2018). This is due not only to their capability for surface modification with targeting ligands but also to some of their other intrinsic properties, such as small size, controlled drug release profiles, extended circulation times, improved drug stability, and enhanced bioavailability (Mishra et al., 2023; Correia et al., 2022; Khare et al., 2023). Compared to other types of nanocarriers, LNPs present higher biocompatibility and greater encapsulation efficiencies (Khare et al., 2023). They also achieve elevated transfection rates due to their remarkable fusogenic properties (Veiga et al., 2023), mostly because of their similarity to natural membranes (Menon et al., 2022). Due to these characteristics, LNPs are allegedly more likely to be taken up by the brain (Neves et al., 2021), even without any functionalization (Arduino et al., 2020). However, some authors argue that this passive strategy is difficult to control (Pucci et al., 2020). Therefore, the potential of active targeting of LNPs emerges as a more promising delivery strategy.

Despite extensive literature and academic production, a significant gap remains between emerging research and the clinical application of active targeting of LNPs. Key challenges include characterizing surface density, streamlining formulation screening, enhancing stability, ensuring scale-up feasibility, among others (Menon et al., 2022; Shan et al., 2022). Specifically, selecting the appropriate ligand is crucial and challenging in the development of actively targeted nanoparticles for circumventing the BBB. This complexity is partly due to the difficulty of comparing studies, which often involve several variables (Zhang et al., 2021). Among the aforementioned scientific literature, numerous available reviews focus on functionalization strategies for overcoming the BBB (e.g., (Mishra et al., 2023; Teixeira et al., 2023; Correia et al., 2022; Khare et al., 2023; Ding et al., 2020; Guyon et al., 2020; Sapsford et al., 2013). Some of the available reviews usually present functionalization alternatives within a broad overview, highlighting the diversity in the field. These valuable works tend to focus on listing and explaining different approaches rather than on a comparative analysis of outcomes or in-depth discussion of study specifics. Consequently, there is an opportunity for an updated, comprehensive analysis that builds upon these foundations. Such kind of comparative analysis could provide insights into which strategies have shown the most promising results within their respective experimental designs, thereby offering an indication of the relative success of each approach.

This review focuses on the innovative surface modification strategies of LNPs aimed at enhancing BBB penetration. These strategies range from small molecules and natural compounds to complex biomolecules like peptides, proteins, and antibodies, including combined approaches. Distinguishing itself from previous works, our review aims to provide a comparative analysis of their effectiveness in enhancing BBB penetration compared to their respective unfunctionalized nanoparticles. By highlighting study characteristics that allow for comparisons of the relative effectiveness of various combinations, we were able to draw direct conclusions. Additionally, we also point out features that, if widely adopted, could enable more precise future comparisons. This

work seeks to critically assess whether the currently available data can determine which functionalization strategy might be a more promising candidate, or if some particular methodological approaches could facilitate such conclusions in the future. Understanding these aspects is crucial for future research and application in drug delivery systems for CNS disorders.

2. Bibliometric study

2.1. Methodology

Our search included the Science Direct and PubMed databases, initially conducted on February 16th, 2022, and last updated on July 31st, 2024. We searched for text word terms including: ("functionalized" or "conjugated" or "modified") and "lipid nanoparticles" and "blood brain barrier". We adapted the standards and principles of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021), to suit the specific context of the publications within this review. For our eligibility criteria, we included research articles published after 2016 that contained a strategy for evaluating nanoparticles, either *in vitro* or *in vivo*, in terms of their ability to overcome the BBB or enhance drug concentrations in brain tissue. Studies were excluded if they involved drug administration via intranasal, intracranial, pulmonary, or oral routes, or if the formulation was in a pharmaceutical preparation other than lipid nanoparticles. Hybrid nanoparticles were considered if their primary composition and manufacturing method aligned with the context of LNPs.

The first evaluation in the selection process was reviewing titles and abstracts. We designed a pre-defined data collection form in Microsoft Excel® with the information to be extracted during the full text reviewing: author, type of lipid nanoparticle, functionalization category, functionalization strategy, model of evaluation, encapsulated drug, therapeutic goal, size and zeta potential (of fully functionalized nanoparticle), results (changes in permeability or drug delivery), and any other relevant results.

When available, we also included the statistical significance as reported by the authors. In total, 42 studies allowed to obtain at least one value for the permeation enhancement ratio. Some studies allowed to obtain more than one ratio due to the combination of *in vivo* and *in vitro* evaluation strategies or due to comparison of more than one evaluation strategy.

We compiled these ratios into a database along with study-specific formulation variables like particle size, zeta potential, functionalization moiety, functionalization category, type of BBB model, and specific model. These data were further analysed using Minitab® Statistical Software 21.4.3.0. We conducted a Principal Component Analysis (PCA) with a correlation matrix to explore data patterns and variable correlations. We used loading and score plots to visualize data correlations. To assess significance, we examined eigenvalues and utilized scree plots. Additionally, we used an outlier plot to discard the influence of outliers on the conclusion. Furthermore, a Classification and Regression Tree (CART)® analysis quantified the relative influence of the selected variables and helped visualize additional variability. Finally, variance and main effects analysis further demonstrated different responses among the selected variables.

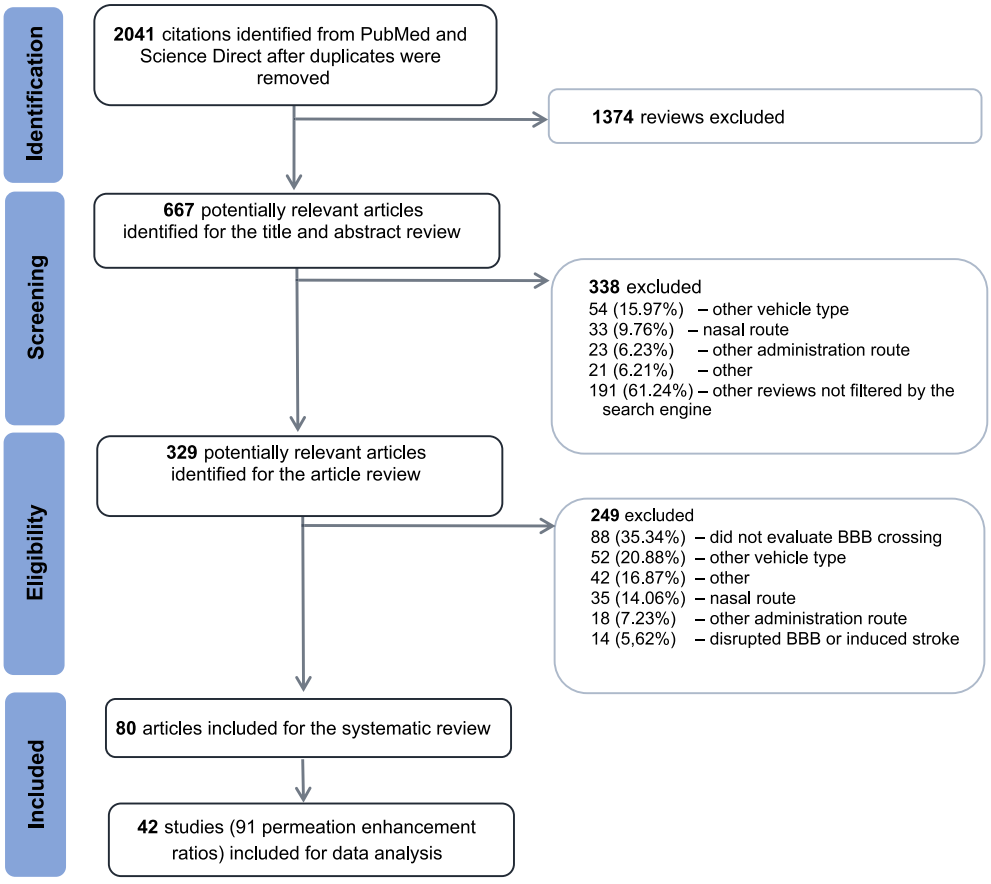

Fig. 1. Flowchart of systematic literature review. Initially, 80 research papers were selected for the systematic review; with 42 fit for data analyses focused on evaluating data correlations and their impact on permeation enhancement ratios. Besides excluding other reviews, the primary reasons for study exclusions were the use of other vehicle type, administration through the nasal route and the absence of an evaluation strategy to assess BBB passage.

2.2. Bibliometric study results

Fig. 1 illustrates the process conducted to identify the 80 studies included for data extraction on functionalization strategies to overcome the BBB, as well as 42 studies for data comparison. We excluded studies that, although demonstrating therapeutic efficacy in evaluation models (e.g., studies: (Kaur et al., 2018; Barbosa et al., 2023; Di Filippo et al., 2022; Scioli Montoto et al., 2018) did not directly measure changes in brain concentrations. Such studies are notable for assessing therapeutic utility, but since their efficacy cannot be uniquely attributed to BBB permeability or due to systemic influence, they fell outside the scope of our review. Studies involving direct administration within the BBB (e.g., study (Byrnes et al., 2023) were excluded. Similarly, studies where the BBB was mechanically or chemically disrupted (e.g., studies (Nong et al., 2023; Hatami Nemati et al., 2023; Sun et al., 2022; Li et al., 2023; Zhang et al., 2023; Nong et al., 2024) were also excluded based on the premise that results from these methods might not be directly applicable to systemic administration. In addition, a considerable number of studies did not meet our eligibility criteria due to their focus on the nasal route (68 in total). While these studies fall outside the scope of this review, we acknowledge the significance of exploring the nasal route for brain drug delivery.

All 80 publications reviewed were categorized as shown in Fig. 2, where peptides, proteins, and antibodies emerged as the most used strategies. All categories are analyzed in the subsequent sections, and the principal features of the studies within each category are presented in their respective tables. The last category (No functionalization/standard modifications) encompasses studies that either do not employ any functionalization methods or utilize surfactants and PEGylation as their chosen strategies. This classification is established since surfactants and PEGylation are regularly used in diverse nanoparticle formulations. Their frequent utilization implies that many other functionalization strategies incorporate PEG or surfactants. This is observed in most of the studies listed across the other categories, thereby justifying their classification under this last category.

Another important criterion in our analysis was the execution of an evaluation method, either *in vitro* or *in vivo*, for assessing changes in nanoparticle and drug passage across the BBB. Among the 80 reviewed studies, the most prevalent method was *in vivo* models, primarily rat and mouse models. Notably, 15 studies combined both *in vivo* and *in vitro* models in the same publication (see Fig. 3).

3. Overview of study results by functionalization category

Each functionalization category is examined to understand its relative effectiveness and unique contributions to the study of BBB permeability enhancement. This section presents a critical evaluation of each stud results. It also highlights emerging trends and potential gaps in current research, paving the way for future innovations in CNS drug delivery systems.

Typically, the selection of a specific functionalization strategy for brain delivery aims to target a particular moiety that is either exclusively present or overexpressed on the BBB (Teixeira et al., 2023). This approach predominantly achieves transportation through receptor-mediated transcytosis (Ding et al., 2020). For instance, the transferrin receptor is widely expressed in ECs and is implicated in the passage of transferrin, a glycoprotein involved in iron transport through the body (Teixeira et al., 2023). This receptor can be targeted using peptides (Neves et al., 2021; Arduino et al., 2020; Sela et al., 2023; Pinheiro et al., 2020; Jain et al., 2023; Kuo et al., 2020), proteins (Muntoni et al., 2019) or antibodies (Marino et al., 2019; Loureiro et al., 2017; Wu et al., 2019). Other examples include insulin and folate receptors, which are highly expressed on ECs and play a vital role in the provision of essential nutrients for brain metabolism (1). The rationale behind the selection of each ligand included in this review is well documented in the scientific literature. Readers are encouraged to consult published reviews that focus on describing various ligands. Additionally, most of the studies listed in Tables 1–5 provide their own specific rationale, complementing the theoretical basis of each approach. Given the scope and breadth of this review, we will not explore the biochemical basis for selecting a given strategy. Instead, we will concentrate on assessing the relative efficacy of each strategy and analyzing the potential for comparison.

3.1. Peptide-modified LNPs

Peptides, composed by a sequence of amino acids, possess unique features that placed them between small molecules and proteins (Sánchez-Navarro et al., 2017; Wang et al., 2022). As small fragments of

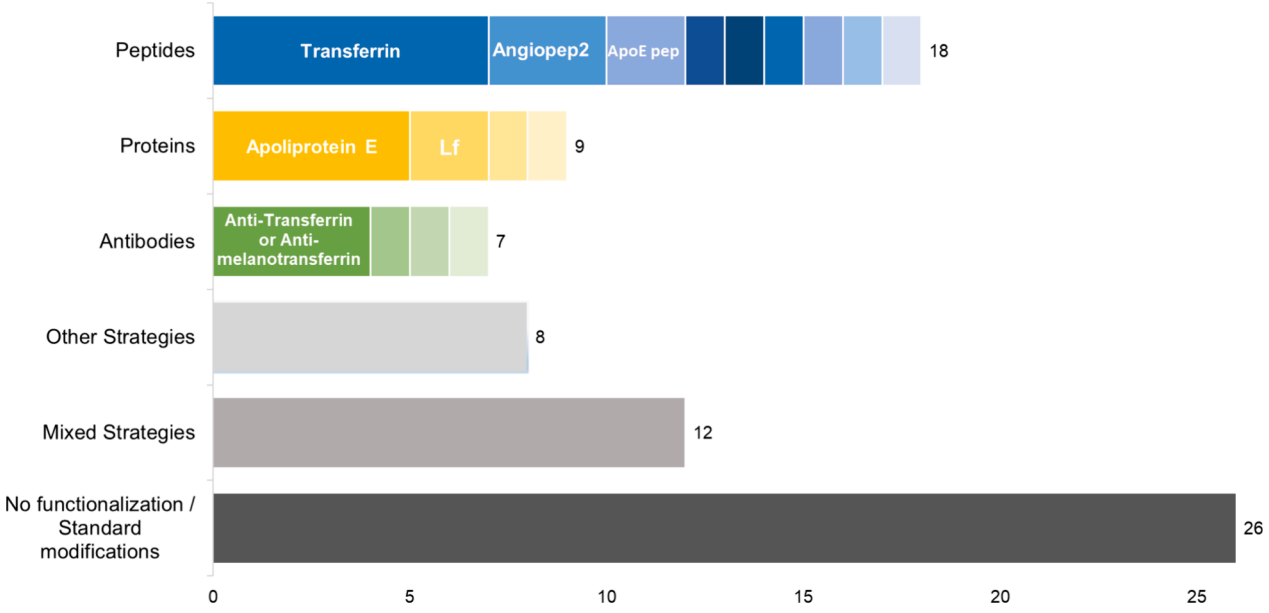

Fig. 2. Categories of Functionalization Strategies in Lipid Nanoparticles for Overcoming the BBB. The most prevalent strategies in the main categories (peptides, proteins and antibodies), are highlighted. Refer to Tables 1 to 5, and S1, for detailed information on the employed strategies in each study. ApoE: ApolipoproteinE; Lf: lactoferrin.

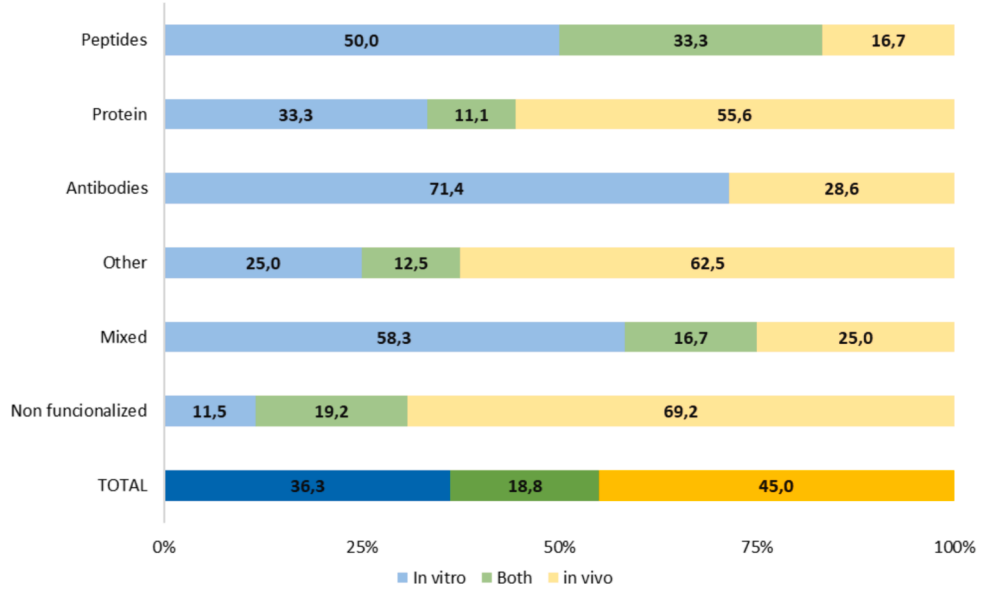

Fig. 3. Distribution of Study Types by Functionalization Category. The figure shows the percentage of studies employing *in vitro*, *in vivo*, or a combination of both evaluation methods across different functionalization categories of lipid nanoparticles. The categories of proteins, other and unfunctionalized show higher prevalence of *in vivo* methodologies.

proteins, they regulate protein interactions with specific receptor sites (Apostolopoulos et al., 2021). Their use as a modification strategy of LNPs has reported some advantages over other functionalization strategies, such as high stability, low immunogenicity, and relatively simple production methods. However, they may show lower target affinity or selectivity and fast systemic clearance (Pucci et al., 2020; Apostolopoulos et al., 2021; Accardo et al., 2013). Several peptide-based modified LNPs have been developed as a strategy to overcome the BBB, as shown in Table 1. The results in this table reveal a trend of increased drug or nanoparticle permeability with peptide functionalization compared to unfunctionalized values in most of the listed studies. However, apart from functionalization itself, the extent of permeability enhancement could also be influenced by various factors. These factors include the evaluation model used in the research, the choice of reference controls, the nanoparticle composition, and the baseline permeability exhibited by the free drug. For instance, Fig. 4 illustrates how the magnitude of permeation enhancement may be affected by the nature of the lipid nanoparticle (Fig. 4-A) or the baseline permeability of each individual drug (Fig. 4-B).

Regarding the evaluation model, Table 1 and Fig. 3 shows a notable prevalence of *in vitro* models for the peptide category. Although six studies combined both strategies, three of them did not quantify the specific changes in permeability for at least one of the models. As a result, only three studies allowed an *in vivo* – *in vitro* correlation (IVIVC). Consequently, it is difficult to derive a generalized conclusion. Studies from Arora and Singh, 2021 and Kato et al., 2023 showed reasonably similar results. They demonstrated a relatively good IVIVC of 1.3–1.5-fold, versus 2-fold, and 5–6-fold versus 3.9-fold, respectively. Results from the study by Dal Magro et al., 2017 were contradictory. The study showed a particularly high permeability enhancement in the *in vitro* model that did not correlate with the *in vivo* results, where no permeability increase was reported. Functionalization did not improved brain passage after intravenous administration route but showed promising results for the nasal route. However, it is important to remark that cell cultures and animal models were not the same across these publications.

There are also examples of negative results in the *in vitro* evaluations of BBB permeability. For example, the results reported by Pinheiro et al., 2020 showed that the inclusion of transferrin might have reduce the permeability of their NLCs. They hypothesized that this reduction might be due to receptor desensitization induced by the quantity of transferrin. They pointed to literature that claims that endogenous transferrin can, in fact, caused saturation of the receptors (Loureiro et al., 2015). In the case of the study conducted by Arduino et al., 2020 using the same transferrin targeting peptide, their nanoparticles failed to cross to the lower compartment of their cellular model (the equivalent to the brain side of the BBB). However, they tended to accumulate inside the endothelial cells and successfully targeted the expression of MC11 transporters inside the cells. Thus, despite no increase in permeability, their affinity for brain endothelial cells could still be exploited as an advantage for future therapies. Analogous assumptions could be made for other studies excluded from the main results of this review because they did not include a barrier model. These studies directly tested and probed the affinity of their nanoparticles in a traditional cell culture of hCMEC/D3, like the one carried out by Gomes et al., 2017.

Another noteworthy finding from the analysis of Table 1 is the BBB permeability comparison of nanostructured lipid carriers (NLCs) and solid lipid nanoparticles (SLNs). All the studies listed on Table 1 that evaluate both types of nanoparticles arrived at the same conclusion: with and without functionalization, NLCs showed higher permeability results than SLNs. The authors suggest that the higher results observed for NLCs compared to SLNs could be explained by changes in nanoparticle crystallinity, conjugation capacity, and also better drug loading/release properties (Neves et al., 2021; Pinheiro et al., 2020; Pinheiro et al., 2020). An example of these differences between NLCs and SLNs is illustrated in Fig. 4-A.

Finally, regarding the physicochemical properties of lipid nanoparticles, it is noteworthy that reported sizes of the functionalized nanoparticles mostly range between 100 and 250 nm. Tables 1 to 5 presents physicochemical the values for the functionalized nanoparticles encapsulating the active pharmaceutical ingredient (API). Negative zeta potential values are predominant. Indeed, in 11 of the 18 studies, they reported zeta potential values between −3.5 and −54 mV. When positive values were observed, they were of relatively low magnitudes (0.83–20 mV) (Arora and Singh, 2021).

The study carried out by Bi et al., 2023 exhibited a notable difference compared to the other studies listed in Table 1. Firstly, they incorporated peptides as lipopolymers containing polysarcosine as an alternative to PEGylated lipids. Their aim was to achieve less immunogenicity while retaining the stealth and stabilization properties typically conferred by PEGylation. They found that as the length of the polysarcosine chain or

Table 1
Comparative analysis of key study features for peptide-modified lipid nanoparticles to overcome the BBB.

Study	Type of lipid nanoparticle	Particle size; Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
Neves et al. (2021)	SLNs and NLCs	SLNs 162 ± 6 nm; −29 ± 5 mV NLCs 183 ± 12 nm; −21 ± 2 mV	Transferrin targeting peptide (Tf)	Curcumin	***In vitro*** hCMEC/D3	In both systems (NLCs and SLNs), the permeability profile increased by **1.5**-fold with Tf functionalization. Permeability calculations were based on drug concentrations measured in the luminal and abluminal chambers over 4-hour period. For both functionalized and plain samples, **NLCs** showed **higher permeability** than SLNs (see Fig. 4A).	The free curcumin results were higher than those encapsulated in plain NLCs and SLNs, but slightly lower compared to the functionalized systems.
Arduino et al. (2020)	NLCs	118.4 ± 2.1 nm; −38.6 ± 0.9 mV	Transferrin targeting peptide (Tf)	Anti-Alzheimer disease ligand: MC111	***In vitro***h CMEC/D3	According to the permeability coefficient, fluorescent pattern of NPs and drug distributions, the authors claim that in this specific case, the Tf functionalization **limited** the delivery of NLCs to the lower compartment of the model. Only unfunctionalized NLCs crosses into the lower compartment of the cellular model (which corresponds to the CNS).	Delivering MC111 in NLCs increased the expression of P-gp and BCRP proteins on endothelial cells of the BBB model, which was the expected result of MC111 delivery. Particularly, the functionalization of NLCs with Tf showed the highest effectivity.
Pinheiro et al. (2020)	SLNs and NLCs	SLNs: 234 ± 18 nm −32 ± 8 mV NLCs: 219 ± 13 nm; −28 ± 2 mV	Transferrin targeting peptide (Tf)	Quercetin	***In vitro*** hCMEC/D3	**NLCs** showed **higher permeability** than SLNs*. Authors concluded that there was **no increase** in permeability due to transferrin functionalization†. Calculations were based on NPs quantification in the abluminal chamber by fluorescence analysis over 4-hour period.	The amyloid-beta studies demonstrated that the quercetin-loaded nanoparticles successfully inhibited fibril formation and decrease peptide aggregation, avoiding an aggregation effect of unloaded nanoparticles. This efficacy was particularly higher in Tf-NLCs, compared to Tf-SLNs and un-functionalized nanoparticles.
Jain et al. (2023)	NLCs	84 ± 9 nm; −3.5 ± 0.9 mV	Transferrin targeting peptide (Tf)	i. Rivastigmine ii Resveratrol	***In vivo*** Wistar rats (female)	Functionalization with transferrin led to a **1.7**-fold*** increase in brain uptake compared to a unfunctionalized NLCs, and a 2.9§ increase compared to free fluorescent dye. Uptake calculations were performed by analyzing brain tissue via confocal microscopy, with animals being sacrificed 6 h post-injection.	Lyophilized samples of the nanoparticles were evaluated up to 6 months under different storage conditions.
Sela et al. (2023)	Liposomes	113.5 ± 1.5 nm; Zeta potential was not reported	Transferrin targeting peptide (Tf)	Monoclonal antibody SynO4[1]	***In vitro*** BMECs ***In vivo*** mice C57/6JOlaHsd (female)	Tf-functionalized liposomes crossed the BBB model, showing increasing penetration over a 24-hour period (*in vitro* results did not include a comparison with unfunctionalized liposomes). In mice with an induced Parkinson's disease model, the accumulation of functionalized liposomes was **3**-fold#	In the induced Parkinson's disease model, administering Tf-liposomes decelerated disease progression, lessened both intracellular and extracellular alpha-synuclein aggregation, and mitigated neuroinflammation. The authors also proved that Tf-liposomes led to enhancements in motor

(*continued on next page*)

Table 1 (*continued*)

Study	Type of lipid nanoparticle	Particle size; Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
						higher compared to unfunctionalized liposomes, while the antibody content in brain tissue was 2.3-fold higher for the same comparison. Accumulation calculations were performed by imaging extracted organs using *in vivo* imaging system, with animals being sacrificed 12 h post-injection.	functions and cognitive capabilities of treated mice.
Kuo et al. (2020)	Dual phase SLNs	Between 174.3 and 205.47 nm; About −42 and −30 mV	Transferrin targeting peptide (Tf)	i Curcumin ii Quercitin iii Rosmarinic acid iv Nerve growth factor	***In vitro*** HCVP/hCMEC/HA	The functionalized NP showed an increase in permeability for both free drug and unfunctionalized NP (in the latter case up to **3**-fold* & **). Loaded unfunctionalized nanoparticles showed a decrease in free drug permeability for curcumin and quercetin, and a slightly increase for rosmarinic acid and nerve growth factor (see Fig. 4B). Permeability calculations were based on drug concentrations measured in the luminal and abluminal chambers over 4-hour period	Each drug was encapsulated in different formulations for permeation studies, and in a single formulation for evaluation of proliferations and protective effect studies.Encapsulation reduced the cytotoxicity of the evaluated drugs. The combined effect of all encapsulated drugs showed an effect in protecting degenerated SK-N-MC cells. The formulation also included cardiolipin to improve affinity to β-amyloid peptide.In their formulation studies, the authors explored different HLB lipid values and other formulations variables and their effects on nanoparticle properties.
Mendes et al. (2024)	NLC	61 ± 1 nm; −18 ± 2 mV	Transferrin targeting peptide (Tf) [2]	Celecoxib	***In vitro*** hBMECs	Transferrin targeting peptide increased the apparent permeability of unmodified NLC by **1.5**-fold*. Permeability calculations were based on celecoxib measurements in the abluminal chamber over a 4-hour period.	Authors conclude that targeting molecules protects NLCs from protein corona formation. In addition, they found that cell-penetrating peptides reduce the ability of unmodified NLCs to cross the cellular model of the BBB. They also compared internalization pathways in HBMECs and U87 cell lines, and finally suggest that a dual functionalization approach optimizes glioblastoma targeting by enhancing BBB crossing and tumor specificity.
Pucci et al. (2020)	Lipid based nanovectors[3]	179 ± 3 nm; −39.0 ± 0.8 mV	Angiopep2	nutlin-3a (rebemadlin) Iron oxide nanoparticles	***In vitro*** hCMEC/D3 +astrocytes +U87MG +SH-SY5Yd	The nanovector concentration crossing the BBB model, calculated by fluorescence intensity, was **1.19** higher* compared to unfunctionalized nanovectors.	Targeting capacity for U87MG and SH-SY5Yd cells before and after passing the hCMEC/D3. The penetration capacity remains unchanged after passing through the barrier culture. Compared to unmodified cores, the functionalization increased BBB permeability and targeting capacity for U87MGcells, and showed no effect for SH-SY5Yd cells. Authors proved the *in vitro* chemotherapeutic efficacy of the nanovectors, including magnetic stimulation.

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Table 1 (*continued*)

Study	Type of lipid nanoparticle	Particle size; Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
Kadari et al., (2018)	SLNs	111.4 ± 3.2 nm; −16.4 ± 1.2 mV	Angiopep2	Docetaxel	***In vivo*** Tissue distribution in male C57BL/6 mice	The accumulation of docetaxel in brain tissue after one hour was **2.44** times higher[§] when comparing functionalized and unfunctionalized SLNs, and 4.07 times higher compared to free drug administration. Real-time fluorescence imaging showed a significant increase in intracranial intensity with functionalized SLNs.	*In vitro* apoptosis studies in U87MG and GL261 cells showed better efficiency for decorated SLNs compared to free drug and plain SLNs. The antiglioma efficacy of the formulations was evaluated in an animal model (mice) where functionalized formulations showed a significantly higher survival time than plain SLNs and free drug.
Du et al. (2024)	Liposomes	About 120 nm; Zeta potential was not reported	Angiopep2[4]	None	***In vitro*** bEnd.3 ***In vivo*** ICR mice	Authors found that the length of the polyethylene glycol (PEG) chain in Angiopep2 modified liposomes influence their ability to cross the BBB. Both *in vitro* and *in vivo* results showed higher accumulation for liposomes with longer PEG chains. Results did no included comparison with unmodified liposomes.	Contrary to the observed with the BBB *in vitro* crossing, liposome internalization in U87MG cells was higher for shorter PEG chains. *In vivo* tumor accumulation also correlated with PEG chain length, showing higher accumulation for longer PEG chains.
Dal Magro et al. (2017)	SLNs	119.7 ± 2.5 nm; −54.3 ± 2.1 mV	Apolipoprotein E-derived peptide	None	***In vitro*** hCMEC/D3 ***In vivo*** Male Balb/c mice (biodistribution)	***In vitro*** Functionalization with the Apo-E derived peptide increased permeability in the studied model by about **9.5** to **14** times** compared to control SLNs. Permeability calculations were based on radioactivity measurements in the abluminal chamber over a three-hour period ***In vivo*** Functionalization did **not improve** brain accumulation in the **intravenous** administration route. Intraperitoneal injection showed no accumulation with or without modification. Accumulation assessments were performed using fluorescent molecular tomography.	Internalization studies suggested that the primary mechanism by which SLNs-mApoE entered hCMEC/D3 was through a clathrin-mediated endocytosis. For intravenous administration route, the brain nanoparticle accumulation after 1 h was 0.67 % for plain SLNs and 0.15 % for ApoE-functionalized SLNs. After 4 h, the accumulation was 0.15 % for plain SLNs and 0.05 % for functionalized. On the other hand, the intratracheal instillation administration route reached brain concentrations equivalent to 1.38 % (plain) and 4.53 % (functionalized) after 1 h, and 0.36 % (plain) and 1.75 % (functionalized) after 24 h.Inflammatory reactions did not increase in mice treated with nanoparticle formulations compared to control (saline solution).
Kato et al. (2023)	Liposomes	Between 76.1 ± 2.9 and 84.5 ± 6.6 nm; Between −0.16 ± 1.35 and 0.825 ± 1.08 mV	Apolipoprotein E mimetic peptide	None	***In vitro*** hCMEC/D3 ***In vivo*** mice ddY (male)	In the *in vitro* model, functionalization with 0.25 % mol, 0.5 % mol, and 1 % mol of Apolipoprotein E mimetic peptide enhanced the permeability of plain liposomes by approximately **5 to 6**-fold***. This increase was measured by fluorescent intensity in the abluminal chamber after 24 h. *In vivo* results shows brain accumulation of **3.9**-fold**, measured using	Before focusing on peptide-modified liposomes, the authors assessed the BBB *in vitro* permeability of eight different peptides, ultimately selecting the Apolipoprotein E mimetic peptide due to its higher permeability results.In mice, the authors demonstrated the capability of ApoEdp-modified PEGylated liposomes to penetrate beyond the BBB. This was able due to the use of three-dimensional

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Table 1 (*continued*)

Study	Type of lipid nanoparticle	Particle size; Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
						fluorescent intensity of brain homogenate of mice scarified 24 h after injection.	imaging with tissue clearing.
Reginald-Opara et al. (2022)	Liposomes	107.7 ± 8.4 nm; −30.9 ± 1.0 mV	Glutathione	None	***In vitro*** hBMECs	Glutathione covered liposomes **cross** BBB model. **No comparison** without glutathione is included in this work. Further references to the direct effect of glutathione presence are available in previously published works referenced by the authors (Gaillard et al., 2014; Salem et al., 2015; Maussang et al., 2016).	This study describes transport mechanisms related to pH responsiveness of the lipid core.Their main conclusion is that only the non pH-responsive lipid core were exocytosed from the cells as whole particles.
Pinheiro et al. (2020)	SLNs and NLCs	SLNs 201 ± 23 nm; −25 ± 7 mV NLCs 222 ± 22 nm; −25 ± 3 mV	Rabies virus glycoprotein (RVG)	Quercetin	***In vitro*** hCMEC/D3	Compared to their respective unfunctionalized nanoparticles, RVG functionalization resulted in a **1.5**-fold* increase in the permeation of both SLNs and NLCs into the abluminal chamber, measure by flouresence intensity over 4-hour period. **NLCs** showed **higher permeability** than SLNs. The permeability of plain NLCs was comparable to that of SLNs-RVG.	In both NLCs and SLNs, functionalization improved beta-amyloid inhibition (thioflavin T binding inhibition assay) compared to the plain nanoparticles. The results of RVG-NLCs and RVG-SLNs were comparable to free drug inhibition.
Tang et al. (2024)	Lipid nanoparticles[3]	130.1 ± 4.84 nm; Approx. + 37 mV	DAT peptide (based on transmembrane peptide DP7)	siRNA	***In vitro*** bEnd.3 ***In vivo*** female C57BL/6 J mice	Functionalization with DAT enhanced NPs permeability across the BBB model by approximately **1.5**-fold**. This increase was measured by the changes in fluorescence intensity of GL261 cells cultured in the abluminal chamber. Ex vivo analysis of brain tissue 4 h after administration revealed minimal accumulation for naked siRNA. There was an increased signal for the LNPs and a higger signal for the DAT-modified LNPs. Fluorescence measures were unquantified.	Authors demonstrated that their formulation effectively targets brain tumors and holds promise for glioma immunotherapy. They also evaluated the pathways for peptide internalization, transfection efficiency, endosomal escape, and gene silencing efficacy
Arora and Singh (2021)	Liposomes	Specific reported values range between: 141.3 ± 17.25 nm and 190.8 ± 42.21 nm; 13.1 ± 2.3 mV and 20.5 ± 3.0 mV	i Penetratin (Pen) ii Chimeric rabies virus glycoprotein fragment (RVG9R) iii Rabies virus derived peptide (RDP) iv CGN peptide v Mannose (MAN)	Plasmid encoding VGF protein	***In vitro*** bEnd.3 cells, primary rat glial, and primary rat neuronal cells. ***In vivo*** female C57BL/6 mice (Evaluation of VGF expression).	***In vitro*** RVG9R-MAN, RDP-MAN and CGN-MAN modified liposomes increased the transport of plain liposomes by **1.4 to 1.8***. The others showed no difference compared to plain liposomes, crossing at approximately 10 %. Calculations were based on NPs quantification in the abluminal chamber by fluorescence analysis over 24 h ***In vivo*** Pen-MAN, RDP-MAN and RVGR9-MAN increased	Tissue observations did not reveal any signs of toxicity from the liposome formulations in the brain, liver, spleen, kidney, heart, or lungs.

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Table 1 (*continued*)

Study	Type of lipid nanoparticle	Particle size; Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
						VGF expression in the brain by **2**-fold* compared to plain liposomes, saline treatment, and the rest of liposomes. Transfection efficacy was evaluated on the 6th day after administration.	
Bi et al. (2023)	Liposomes	Between 110 and 130 nm; Between 25 and 35 mV	Polysarcosine[6]	mRNA	***In vivo*** Zebra fish	The administration in polysarcosine-liposomes with shorter carbon length (14 carbons) and polysarcone length (2 k) resulted in a **4**** to 6-fold*** increase in protein expression in zebrafish embryos.	The polysarcosine-modified nanoparticles exhibited superior *in vitro* and *in vivo* transfection rates compared to the PEGylated nanoparticles, particularly showing higher rates for lipids with a 14-carbon chain length with shorter lipophilic chain (2 k). Concerning extended circulation times, lipids with longer carbon chains (18 carbons) and longer lipophilic chain (2 k) proved to be more effective than PEGylation, surpassing shorter carbon chains in efficiency.
Arora et al. (2020)	Liposomes	Specific reported values range between: 136.0 ± 6.9 nm and 178.5 ± 2.3 nm; 8.0 ± 1.2 mV and 17.0 ± 2.0 mV	i Penetratin (Pen) ii Rabies virus glycoprotein (RVG) iii Mannose (MAN) iv Cell penetrating peptide	Brain-Derived Neurotrophic Factor Gene	***In vitro*** bEnd.3 cells, primary rat glial, female C57BL/6 mice	*In vitro*, RVG-MAN and PEN-MAN modified liposomes increased transport of plain liposomes by **1.8 to 2***. Transportation was measure by fluorescent intensity in the abluminal chamber. *In vivo*, RVG-man and PEN-MAN increased brain distribution of lissamine rhodamine dye by approximately **1.5**-fold and **2**-fold*, respectively, compared to plain liposomes. No significant differences were observed with MAN, PEN and RVG only modified liposomes in both type of models. All formulations included also cell penetrating peptide.	Additionally, the authors assessed the biocompatibility of their liposomes and their cellular uptake. They also measured transfection efficiency in both *in vitro* and *in vivo* settings, observing higher efficiency in RVG-MAN and PEN-MAN modified liposomes.

Notes:

Abbreviations: Apo: apolipoprotein; BBB: Blood-brain barrier; Bend.3: Brain endothelial cells derived from mice; CNS: Central nervous system; HA: Human astrocytes; hBMECs: human brain microvascular endothelial cells; HBVP: Human brain vascular pericytes; hCMEC/D3: Human cerebral endothelial cells; HLB: hydrophilic-lipophilic balance; LNPs: lipid nanoparticles; MAN: Mannose; NLCs: Nanostructured lipid carriers; NP: Nanoparticles; PEG: polyethylene glycol; Pen: Penetratin; RDP: Rabies virus-derived peptide; mRNA: Messenger ribonucleic acid; RVG: rabies virus glycoprotein; siRNA: small interfering ribonucleic acid; SLNs: Solid lipid nanoparticles; Tf: Transferrin targeting peptide; VGF: nerve growth factor.

Reported statistically significant permeability improvements (p value: * < 0.05; ** < 0.01; *** < 0.001; $^{\#} < 0.0005$; $^{\S} < 0.0001$).

† Authors report no statistically significant change in permeability (p value ≥ 0.05).

1- Monoclonal antibody targeting alpha-synuclein aggregation (encapsulated drug), which is a signature characteristic of Parkinson's disease progression.

2- Authors also include functionalization with 5 different types of cell penetrating peptides and tumor targeting peptide (RGFfK) for glioma targeting.

3- Not categorized, possibly corresponds to SLN.

4- Authors evaluate different Angiopep2 coated liposomes varying the length of PEG chain included in the formulation of liposomes.

5- This study reports the result of ten different formulations from five different functionalization alternatives proposed by the authors: plain liposomes, five corresponding to each of the individual alternatives, and four combinations with mannose (Pen-MAN, CGN-MAN, RVGR9-MAN and RDP-MAN). Specific values for particle size and zeta potential for each combination can be found in the respective reference.

6- Polysarcosine was incorporated to the nanoparticles as peptide-lipid lipopolymer. Several lengths of the lipid chain were evaluated in this publication as alternatives for the use of PEGylated lipids.

Table 2
Comparative analysis of key study features for protein-modified lipid nanoparticles to overcome the BBB.

Study	Type of lipid nanoparticle	Particle size;Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
Neves et al. (2017)	SLNs	SLNs-Palmitate-ApoE: 174.2 ± 10.3 nm; −11.46 ± 2.56 mV SLNs-DSPE-ApoE: 164.8 ± 22.2 nm; −11.46 ± 2.56 mV	Apolipoprotein E (ApoE)	None	***In vitro*** hCMEC/D3	The apparent permeability coefficient increased by 1.4 times for SLNs-Palmitate and by **1.5*** times for SLNs-DSPE when functionalized. The apparent permeability coefficient calculations were based on NPs quantification in the abluminal chamber by fluorescence analysis over 4-hour period. The cellular uptake in hCMEC/D3 model cells showed a 1.8-fold increase for SLNs-Palmitate and a **1.9**-fold increase for SLNs-DSPE compared to their respective unfunctionalized controls. After internalization, the transcytosis of the SLNs was evaluated using various techniques, revealing their passage through the barrier to the basolateral side of the model.	The authors reported that the internalization of SLNs-Palmitate-ApoE and SLNs-DSPE-ApoE predominantly occurs through clathrin-mediated endocytosis, after evaluating of three potential mechanisms for BBB penetration: clathrin-mediated endocytosis, caveolae-mediated endocytosis, and micropinocytosisInternalization studies indicated that these ApoE-functionalized SLNs have the ability to cross the BBB without undergoing significant degradation inside the cells, as no major changes in their properties were detected.
Neves et al. (2016)	SLNs	SLNs-Palmitate-ApoE: 217.1 ± 5.8 nm −13.54 ± 1.60 mV SLNs-DSPE-ApoE: 167.8 ± 19.9 nm; −13.05 ± 4.06 mV	Apolipoprotein E (ApoE)	Resveratrol	***In vitro*** hCMEC/D3	Functionalization with ApoE increased drug permeability by **1.8**-fold* (for both types of lipids) compared to unfunctionalized SLNs and the free drug (similar permeability results for free drug and unfunctionalized SLNs). Calculations were based on drug quantification in the abluminal chamber over 4-hour period.	
Rajora et al. (2017)	Porphyrin-lipid nanoparticles[2]	Discoidal shape: 28 ± 8 nm; Spherical shape: 29 ± 9 nm;Zeta potential was not reported	Apolipoprotein E3 (ApoE3)	None	***In vivo*** U87-GFP orthotopic tumour-bearing mice	Animal studies demonstrated an accumulation ratio of 4:1, favouring tumour tissue over healthy tissue. *In vivo* accumulation of functionalized nanoparticles in the brain accounted for approximately 3 ± 1 % of the total administered dose. This study did not include a unfunctionalized control group to assess brain accumulation of nanoparticles without ApoE3.	The authors conducted a comparative assessment of cellular uptake using U87GM cells and ldlA7 cells (hamster ovary cells characterized by low expression of the LDL receptor, which serves as the target for ApoE3 functionalization). Notably, the cellular uptake of spherical nanoparticles (containing colesteryloleate) was found to be 3 to 4 times higher in U87GM cells compared to ldlA7 cells. In the case of discoidal nanoparticles, uptake increased by 2.3-fold in LDL-expressing cells. The authors further demonstrated that the uptake mechanism of the nanoparticles involved receptor-mediated transcytosis, which could be inhibited by either LDL competition or acetylation of ApoE3 moieties on the nanoparticles. In terms of effectiveness, *in vitro* photodynamic therapy treatment resulted in a 83 % reduction in the viability of u87MG cells.

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Table 2 (*continued*)

Study	Type of lipid nanoparticle	Particle size;Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
Athalye et al. (2024)	Lipid nanoparticles[3]	131.6 ± 1.2 nm; −15.6 ± 0.09 mV	Apolipoprotein E3 (ApoE3)	Levetiracetam	***In vivo*** Wistar rats	Compared to unmodified nanoparticles, ApoE3-modified nanoparticles increased brain concentrations of levetiracetam in **1.26**-fold, after 1 h. Compared to free levetiracetam, unmodified and functionalized NPs enhanced brain concentrations by 2-fold* and 2.59-fold*, respectively.	The authors evaluated the release kinetis of the levetiracetam from their NPs, and their cytotoxic effects on HEK293 cell lines. Additionally, they examined *in vivo* safety by evaluating haemolysis and necrotic effects.
Dal Magro et al., (2018)	Lipid nanoparticles[4]	211.3 ± 3.5 nm; 10 ± 0.7 mV	Apolipoprotein E4 (ApoE4)	None	***In vivo*** Male BALB/c mice	The functionalization with ApoeE4 led to a **3**-fold** higher brain accumulation of nanoparticles at 30 min. Three different concentrations of ApoE4 (5, 10 and 20 μg/mL) were tested. The highest accumulation was observed at the lower ApoE4 concentration (5 μg/mL). Brain accumulation was quantified using immunohistochemistry and fluorescence measurements of brain tissue from animals sacrificed 30 min after administration.	The authors demonstrated in hCMEC/D3 cell culture that the principal mechanism for BBB penetration of the ApoE4-modified nanoparticles was clathrin-mediated endocytosis. In contrast to the findings in brain accumulation, liver and intestine accumulation showed a direct correlation with increasing quantities of ApoE4.
Singh et al. (2016)	SLNs	121.0 ± 5.6 nm; −21.5 ± 1.2 mV	Lactoferrin (Lf)	Docetaxel (DTX)	***In vivo*** Female Swiss albino mice	The area under the curve of docetaxel in the brain was approximately **2.27**-fold* higher compared to unfunctionalized SLNs, over 8-hour observation period. After a one-hour interval, the administration of free docetaxel resulted in brain concentrations comparable to those achieved with functionalized SLNs. However, in contrast to both nanoparticle formulations, the administration of free drug showed no appreciable brain accumulation at 2, 4, or 8 h.	Functionalization of SLNs increased both cellular uptake and apoptosis in studies conducted on U87MG cells. Additionally, both SLNs formulations exhibited higher apoptotic effects compared to the free drug. The encapsulation of docetaxel in SLNs resulted in an increase in its plasma half-life from 1.89 to approximately 2.3 h. The authors also evaluated the stability of the nanoparticles in both normal saline and 10 % serum. The study presents the findings regarding nanoparticle accumulation in various major tissues within the *in vivo* model.
Zhao et al. (2018)	NLCs	170 ± 14 nm; −15.9 ± 1.1 mV	Lactoferrin (Lf)	Nimodipine (NMD)	***In vivo*** Biodistribution on BALB/c nude mice	Although the intensities were not quantified, whole-body imaging revealed that lactoferrin-functionalized NLCs exhibited **higher brain accumulation**, 24 h after administration. The second-highest accumulation was observed with PEGylated but unfunctionalized NLCs, while the least accumulation was observed with unfunctionalized NLCs without PEGylation.	The cellular uptake of lactoferrin-functionalized NLCs in PC12 cells resulted in a 1.5-fold increase in internal fluorescence. However, the authors discovered that beyond a certain Lf content (specifically, higher than > 100 μg/ml), the cellular uptake decreased. *In vitro* serum stability determination was included in the study. Formulations were tested in a mice stroke induction model using sodium nitroprusside. Functionalized NLCs containing nimodipine improved viability by more than 30 %.

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Table 2 (*continued*)

Study	Type of lipid nanoparticle	Particle size;Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
Muntoni et al. (2019)	SLNs	PEGylated linker: Transferrin: 500 ± 45 nm Insulin: 445 ± 41 nm Non PEGylated linker: Transferrin: 437 ± 55 nm Insulin: 429 ± 8 nmZeta potential was not reported	i Transferrin ii Insulin	Didodecyl-methotrexate	***In vitro*** hCMEC/D3 ***In vivo*** Male Wistar rats	*In vitro* results showed that encapsulation itself caused a 3.52-fold* increase in drug permeability compared to free drug. However, when SLNs were functionalized with any of the four different linker/protein combinations, there was a **reduction** in permeability compared to unfunctionalized SLNs, showing a 1.5 to 1.9* increase compared to the free drug. Permeability calculations were based on drug concentrations measured in the abluminal chamber over 24-hour period. SLNs functionalized with insulin or transferrin with a PEGylated linker showed the highest *in vivo* brain accumulation (approximately 1 % of the total dose retained by gram of tissue) compared to functionalized non-PEGylated, unfunctionalized, and free drug. Brain accumulation was determined through prior derivatization and HPLC flourometric analysis.	For all cases, the highest drug accumulation was found in the spleen, liver and lungs. Functionalization increased the accumulation in the above organs regardless of the type of linker or protein used for functionalization.
Kuo et al. (2021)	Liposomes	About approximately 150 to 190 nm; About approximately 18 to −42 mV[5]	Leptin	i Resveratrol ii Epigallocatechin gallate(EGCG)	***In vitro*** HBMECs/HA/HBVPs	For EGCG, encapsulation only increased permeability by about 10-fold compared to the free drug, while the inclusion of leptin increased permeability **2**–fold* compared to plain liposomes. For resveratrol, encapsulation reduced the permeability of the free drug by 50 %. Functionalization increased the permeability of plain liposomes, but it did not represent a significant change compared to free resveratrol†. Permeability coefficients were calculated using drug concentrations measured in the abluminal chamber over 4-hour period	Treatment with the leptin-modified liposomes containing resveratrol and EGCG increased the survival rates of SH-SY5Y cells treated with 1-methyl-4-pyridinium from 67.78 % to more than 80 %. Moreover, the treatment evidenced downregulation of pro-apoptotic factors and upregulation of apoptosis inhibitors.

Notes:

Abbreviations: Apo: apolipoprotein; BBB: Blood-brain barrier; DSPE: 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine;EGCG: Epigallocatechin gallate; HA: Human astrocytes; HBMECs: Human brain microvascular endothelial cells; HBVP: Human brain vascular pericytes; hCMEC/D3: Human cerebral endothelial cells; Lf: Lactoferrin; DTX: Docetaxel; NLCs: Nanostructured lipid carriers; NMD: Nimodipine; NP: Nanoparticles; SLNs: Solid lipid nanoparticles.

Reported statistically significant permeability improvements (p value: * < 0.05; ** < 0.01).

† Authors report no statistically significant change in permeability (p value ≥ 0.05).

1- Tamoxifen was included as a functionalization strategy to enhance brain accumulation due to its inhibitory capacity on multidrug resistance-related proteins (MRPs), which are involved in expelling substances that cross the BBB.

2- The photophysical properties of porphyrins allow their inclusion in theragnostic applications. Two groups of lipid nanoparticles (discoidal and spherical) were prepared. The spherical morphology was a result of a change in the composition due to the inclusion of cholesteryl oleate in the nanoparticles. Spherical forms were the only variant used in the *in vivo* evaluation due to their higher uptake by U87GM cells.
3- Cetyl palmitate nanoparticles were prepared by high-pressure homogenization technique and stabilized with polysorbate 80.
4- The authors reported different values for lipid composition, particularly regarding the percentages of 1,2-distearoyl-*sn*-glycero-3-phosphocholine and dihexadecyl phosphate (DHDP) in the formulation
5- Contrasting with other categories, the investigation of specific internalization and transport pathways is particularly emphasized in protein-functionalized lipid nanoparticle studies (Zhao et al., 2018; Dal Magro et al., 2018; Rajora et al., 2017; Neves et al., 2017). For example, Neves *et al* (Neves et al., 2017) found that clathrin-mediated endocytosis appeared to be the primary route for SLNs internalization. Moreover, SLNs functionalized with ApoE demonstrated increased uptake, likely due to its interaction with LDLR located in clathrin-coated pits on the cell surface.

the lipid carbon tail increased, there was a decrease in transfection efficiency. This decrease was offset by an increase in circulation time. Second, their study included the encapsulation of messenger ribonucleic acid (mRNA). Together with the encapsulation of siRNA in the study by Tang et al., 2024, and the incorporation of Brain-Derived Neurotrophic Factor Gene and a protein-encoding plasmid in the studies from Arora and Singh, 2021; Arora et al., 2020, these are the only studies with gene therapy or RNA therapy among the other listed. Most of the other studies primarily focused on small molecule delivery.

3.2. Protein-modified LNPs

Proteins are a larger and more complex class of biomolecules that possess higher target specificity and stronger affinity than peptides (De La Rica and Matsui, 2010). However, they might present some disadvantages, such as potential immunogenicity (Ilinskaya and Dobrovolskaia, 2016) and increased structural complexity (Spicer et al., 2018). Table 2 illustrates the versatility of protein-modified LNPs in enhancing BBB penetration. Overall, protein functionalization consistently enhanced drug permeability and cellular uptake. However, it is notable that there is no clear-cut or linear trend observed among these studies. Importantly, increasing concentrations of the functionalization agent does not necessarily lead to permeability-increasing effects. For instance, Zhao et al., 2018 found that beyond a specific amount of lactoferrin content, competitive binding to receptors appeared to limit BBB penetration. In their formulation, this threshold was higher than 100 μg/mL, tested up to 500 μg/mL. Dal Magro et al., 2018 observed a similar phenomenon, where the highest brain accumulation occurred at the lowest tested ApoE4 concentration (ranging from 5 to 20 μg/mL). In contrast, they found a direct correlation of higher ApoE4 concentrations and their accumulation in other organs, such as the liver and intestine.

In this category, *in vitro* models remain the predominant method to evaluate permeability. Only one study combined *in vivo* and *in vitro* assessments, and it showed a limited IVIVC. *In vitro* results from Muntoni et al., 2019 used two different proteins (insulin and transferrin) with PEGylated and non-PEGylated linker, combining four different strategies within the same study. All combinations showed lower BBB permeability for functionalized nanoparticles compared to unfunctionalized ones. In contrast, the *in vivo* biodistribution results indicated that functionalized nanoparticles with a PEGylated linker (for both proteins) exhibited higher brain concentrations than all other samples. However, it is worth noting that the achieved brain concentration was relatively low in terms of the total dose (less than 1 %).

In line with our previous observations, nanoparticle composition continues to show a significant influence on permeability, as shown by the results reported by Muntoni et al., 2019 and Kuo et al., 2021. In the latter study, the effects of both encapsulation and functionalization vary based on the cargo. Specifically, while encapsulation alone did not affect the permeability of resveratrol, functionalization reduced it. In contrast, for epigallocatechin gallate, encapsulation increased permeability, and subsequent functionalization further enhanced this effect. Beyond composition, properties like shape also play a role, as evidenced in the study from Rajora et al., 2017. They created lipid nanoparticles in both discoidal and spherical shapes, mirroring the forms of low-density lipoprotein receptor (LDLR) in the brain. Their *in vitro* studies with U87GM and ldlA7 cells (which lack LDLR expression) showed higher uptake of spherical nanoparticles. This was linked to the tertiary structure of apoE3 aligning with the nanoparticles' shape. Notably, these shape changes were achieved by altering the composition, specifically through the addition of cholesteryl oleate.

3.3. Antibody-modified LNPs

Antibodies (Abs) are a unique type of glycoproteins involved in the specialized (adaptative) immune response (Arruebo et al., 2009). Abs become an emerging trend in targeting therapies primarily due to its exceptional specificity (Lu et al., 2020; Marques et al., 2020). One noteworthy observation concerning the studies involving the antibodies listed in Table 3 is the utilization of multiple Abs within the same study. This was done to a greater extent than what was previously observed with multiple peptides or proteins. Some of the accumulation and effectiveness results, mostly showing a cytotoxic effect on cancer cells, are increased when double functionalization is performed. This suggests that the synergistic effect of dual functionalization could be a promising approach to enhance overall efficacy.

For example, Kuo and Lee, 2016; Kuo and Lee, 2016 and Gandomi et al., 2017 featured dual antibody modification in their studies, each with different objectives. In the first case, Kuo and Lee, 2016 targeted the BBB with both Abs, while in the second, they used one antibody for BBB penetration and another for glioblastoma targeting (Kuo and Lee, 2016). Conversely, Gandomi et al., 2017 combined Abs for precise targeting beyond the BBB, rather than focusing on its initial passage. They used Abs against contactin-2 and neurofascin-2, which are present in the myelin sheath of neurons and are targeted by autoimmune responses associated with multiple sclerosis (Gandomi et al., 2017). Encapsulation alone increased drug passage, while functionalization with these two Abs did not improve BBB permeability. In fact, the functionalization decreased the permeability compared with unfunctionalized nanoparticles. This is not surprising since the inclusion of the Ab was intended for myelin targeting.

In a different combination approach, Marino et al., 2019 designed a vehicle with intrinsic paramagnetic properties and modified it with an antibody against transferrin receptor. They demonstrated the synergistic effect of magnetic stimulation to further enhance drug permeation across the BBB, as showed in Fig. 5-B. Although magnetic stimulation had the most significant contribution enhancing permeation, this disruption method is outside the scope of this review. Thus, we consider this particular study focusing on their evaluation of the potential of the encapsulation and the further enhancement of the anti-transferrin effect. The study showed an increase in permeability compared to the control either with or without magnetic stimulation.

Another relevant observation is the evaluation of the impact of NPs composition in the permeability. Two examples can be found in the studies carried out by Kuo and Lee, 2016; Kuo and Chao, 2016. On one hand, they found that increases in tripalmitin content reduce the NPs ability to cross the BBB (Kuo and Chao, 2016). On the other hand, results

Table 3
Comparative analysis of key study features for antibody-modified lipid nanoparticles to overcome the BBB.

Study	Type of lipid nanoparticle	Particle size;Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
Loureiro et al. (2017)	SLNs	254 ± 17 nm; −4.0 ± 0.1 mV	Anti-transferrin: OX26 mAb	i Resveratrol ii Grape extract	***In vitro*** Endothelial cells (derived from umbilical cord) and pericytes	Permeability of the OX26-functionalized SLNs was approximately **4** times higher** than that of the plain SLNs, and about 2 times* higher than the control antibody (Mab LB 509). Permeability calculations were based on fluorescence intensity measured in the abluminal chamber over 2 h period	The amyloid-β aggregation study showed that empty functionalized SLNs promoted aggregation compared to the control. The inclusion of resveratrol and grape extract reduced the accumulation in 26 % and 31 %, respectively.
Wu et al. (2019)	NLCs	14 to 30 nmZeta potential was not reported	Anti-transferrin: OX26 mAb	i Baicalein ii Salvianolic acid	***In vivo*** BALB/c nude Mice, male Sprague Dawley rats, male	In mice, functionalized NLCs was found to be present in the brain region (without quantification), unlike the control groups (unfunctionalized and free drug solutions). In the rat model, functionalization with OX26 resulted in a **1.97**-fold* increase in the 24-hour brain AUC of baicalein and a 2.25 increase* in the maximum concentration compared with unfunctionalized NLCs, while unfunctionalized NLCs and a solution of free baicalein yielded comparable results.	Functionalization increased Tmax and decreased $t_{1/2}$.In an ischemic injury model of oxygen-glucose deprivation, the functionalized NLCs showed a protective activity against damage and exhibited some degree of reperfusion.
Marino et al. (2019)	Lipid paramagnetic nanovectors[2]	101.3 ± 1.1 nm; Zeta potential was not reported	Anti-transferrin receptor + Magnetic stimulation (See diagram in Fig. 5)	Temozolomide	***In vitro*** Brain endothelial cells and astrocytes.	Without magnetic stimulation, the anti-transferrin modification improved the NPs crossing approximately **1.9**-fold* at 72 h, measure by fluorescence intensity. When applying magnetic stimulation, the concentration of functionalized nanovectors was 2.4 times higher* compared to un-functionalized nanovectors. In this particular study, magnetic stimulation made the most significant contribution to enhancing permeation.	Regarding the study on glioblastoma multiforme cells, the lowest survival rates (indicating the best anticancer results) were found in the group treated with temozolomide-functionalized nanoparticles with magnetic treatment (7.7 % of healthy cells), followed by the group treated with functionalized nanoparticles without temozolomide and magnetically treated (49.6 %). The other experimental groups showed a higher content of healthy cells (≥96 %). This study includes an evaluation of the internalization pathways of the nanovectors and a proteomic analysis of the synergic pathways triggered by the combined magnetothermal and chemotherapy treatments.
Kuo and Chao (2016)	SLNs	From approximately 79 to 140 nm; Approx. 18 to 35 mV	Anti-Melanotransferrin (MA)	Etoposide (ETP)	***In vitro*** HBMEC-HA	The permeability coefficient of ETP increased by approximately **5.6**-fold compared to unfunctionalized NP. The effect was evaluated at	Encapsulation of etoposide reduced tits cytotoxicity on HMBMEC, HA and U87MG, but survival decreased as the antibody content was increased.Changes in

(*continued on next page*)

Table 3 (*continued*)

Study	Type of lipid nanoparticle	Particle size;Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
						different MA and lipid concentrations (tripalmitin). The permeability coefficient was determined using etoposide concentration measured with HPLC-UV over 5-hour period.	nanoparticle composition, particularly, an increase in tripalmitin, caused a decrease in the ability to conjugate MA and the loading capacity of etoposide.
Kuo and Lee (2016)	SLNs	From approximately 110 to 170 nm; Approx. –22 to –32 mV	i Anti-Melanotransferrin (MA) ii Anti- aprotinin (Apr)	Doxorubicin (Dox)	***In vitro***HBMEC-HA	Compared to unfunctionalized SLNs, the inclusion of increasing concentrations of Apr incremented the BBB permeability coefficient up to **8**-fold. Further inclusion of MA caused double-functionalized SLNs to show a permeability coefficient about **1.89** higher compared to corresponding SLNs functionalized solely with Apr. Compared to unfunctionalized SLNs, double functionalization caused up to 15-fold increase in permeability. The permeability coefficient was determined using doxorubicin concentration measured with HPLC-UV over 5-hour period.	The lipid proportion of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) also had an impact on permeability. The permeability coefficient of double-functionalized SLNs containing 40 % of DPPC lipid was about 21 % higher compared to those containing 20 % DPPC.Studies on U87MG survival tests showed the best results (higher mortality) for the double-functionalized (40 % DPPC) SLNs.
Kuo and Lee (2016)	SLNs	About approximately 170 to 210 nm; Approx. −17 to –32 mV	i 83–14 monoclonal antibody (8314 Mab) ii Anti-epithelial growth factor receptor (AEGFR)	Etoposide (ETP)	***In vitro*** HBMEC-HA	Compared to unfunctionalized SLNs, surface modification with 8314 MAb-SLNs improved permeability by approximately **5**-fold, and 8314MAb-AEGFR-SLNs by about **4.3**-fold. The permeability coefficient was determined using etoposide concentration measured with HPLC-UV over 5-hour period.	Double functionalized nanoparticles (8314 MAb-AEGFR-SLNs) reduced U87GM viability to 35 %.
Gandomi et al. (2017)	SLNs	anti-Nfasc, 158 ± 19 nm; −8.71 ± 0.46 mV anti-Cntn2 161.7 ± 13 nm; −8.66 ± 0.41 mV	i Anti-Nfasc ii Anti-Cntn2	i Methylprednisolone (MEP), ii Coumarin	***In vivo*** C57BL mice (induced multiple sclerosis mouse model).	Antibody functionalization did **not improve**† the brain accumulation of coumarin. Furthermore, there were no significant differences between the two antibodies. Encapsulation itself enhanced the brain accumulation of coumarin. Compared to free drug, brain accumulation increased 4-fold** using plain SLNs and 1.6-fold for functionalized SLNs.	The cellular uptake of coumarin by U87MG cells was 6 times higher when it was encapsulated in plain SLNs. Functionalization with Anti-Cntn2 increased cellular uptake by 8-fold, while functionalization with Anti-Nfasc resulted in a 4-fold increase compared to the free drug. Encapsulation of MEP and functionalization (with either antibody) improved the viability of U87MG cells. The authors also tested the partial substitution of lipids within their nanoparticle formulation. They found that cellular uptake could be influenced by the lipid composition of the nanoparticles.

Notes:
Abbreviations: 8314 Mab: 83–14 monoclonal antibody; AEGFR: Anti-epithelial growth factor receptor; Apr: Anti-aprotinin; AUC: Area under the curve; BBB: Blood-brain barrier; Cntn2: Contactin-2; DPPC: 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; Dox: Doxorubicin; ETP: Etoposide; HA: Human astrocytes; HBMEC: Human brain microvascular endothelial cells; MA: Anti- Melanotransferrin; ME: Methylprednisolone; Nfasc: Neurofascin; NLCs: Nanostructured lipid carriers; NP: Nanoparticles; SLNs: Solid lipid nanoparticles; $T_{1/2:}$ half-life in plasma; Tmax: time to peak measurement.
Reported statistically significant permeability improvements (p value: * < 0.05; ** < 0.01).
† Authors report no statistically significant change in permeability (p value ≥ 0.05).
1- The effect of each antibody was evaluated separately, and in combination. Methylprednisolone was encapsulated for the physicochemical evaluations and viability assay, while coumarin-6 was used for cellular uptake and brain uptake. The authors claim that the differences in characteristics between these two formulations were not statistically significant.

suggest that NPs with an increase in DPPC lipid content showed higher BBB permeability (Kuo and Lee, 2016). In another study, the group of (Gandomi et al., 2017) found that the internalization of the nanoparticles and brain uptake is influenced by variations in lipid content. These observations on composition could somehow be correlated with the previously mentioned observation that NLCs showed greater BBB permeability than SLNs.

In terms of physicochemical properties, the range of particle sizes observed in the study shows that different sizes were effective in enhancing drug delivery across the BBB. Regarding the zeta potential values, there is a predominance of negative and low-value charges, consistent with the behavior observed in the previous categories. However, one study demonstrates BBB crossing with zeta potential values of + 35 mV.

Table 3 shows fewer *in vivo* data compared to previous categories, with only two studies employing *in vivo* evaluation models. One study showed positive results (Wu et al., 2019) while the other indicated no improvement in brain accumulation from functionalization (Gandomi et al., 2017), as previously noted. Moreover, neither of these *in vivo* studies provided *in vitro* data, impeding any estimation of IVIVC within this category. Nevertheless, the results show the potential for antibody functionalization strategies and the potential for synergistic effects of antibody combinations.

3.4. Other functionalization strategies on LNPs

In addition to the most common functionalization strategies identified in our systematic review, a subgroup of studies explored alternative moieties for functionalization. These included small molecules, natural products, or biomimetic derivatives, as detailed in Table 4. In this functionalization approach, all the studies reported positive results. However, unlike some values observed in other categories, the maximum functionalization enhancement observed was about a 2.2-fold increase.

This subgroup includes some affordable and regularly used compounds in other pharmaceutical applications. Yet they showed potential for functionalizing LNPs for brain delivery, similar to previous categories. Additionally, this category also includes novel strategies as the exploited by McConnell et al., 2019. Their study, along with the study of Ray et al., 2021 (from the mixing strategies category), represent one of only two publications in this review exploring the use of aptamers as functionalization strategy.

Contrary to what was observed in the previous subsections, studies in this category showed a predominance of *in vivo* evaluation methods (6 out of 8). Three of them not only evaluated brain concentrations but also assessed the effect of functionalization on accumulation in other organs, highlighting the selectivity potential of their strategies. The study conducted by Rajamanickam *et al* [92] explored the use of a vitamin E derivate to deliver resveratrol-loaded liposomes to the rat brain. While encapsulation significantly increased resveratrol permeability, functionalization marginally increased brain concentrations but significantly reduced accumulation in other organs. In addition, the study by Banerjee et al., 2016 applied functionalization with a somatostatin analog and achieved no only higher brain accumulation but also higher brain tumor accumulation. Moreover, they evaluated the effects of surface modification on biodistribution patterns.

Another interesting study highlighted in Table 4 is the work by Ma et al., 2020. This study stands out not only for its innovative approach in evaluating various functionalized lipids but also for demonstrating the versatility of their LNPs. Their LNPs are capable of delivering a diverse range of therapeutic agents. From small molecules to antisense oligonucleotides and gene-editing proteins. Each type of cargo had a different formulation and achieved brain accumulation (unquantified). This study stands out as one of the few within this review adopting such a diverse approach and exploiting LNPs potential for gene therapy.

3.5. Mixed combination strategies to functionalize LNPs

Although Tables 1 to 3 already included studies that functionalized LNPs with two peptides (Arora and Singh, 2021), two proteins (Muntoni et al., 2019), or two antibodies (Kuo and Lee, 2016; Kuo and Lee, 2016; Gandomi et al., 2017), those approaches aimed to explore synergies within the same category of functionalization. In contrast, studies listed in Table 5 focus on evaluating LNPs that combine two distinct types of functional categories. These combinations include peptides with natural products, proteins with antibodies, proteins with small molecules, or even biomimetic systems combined with peptides. These strategies show the potential for an inter-functional approach when enhancing NPs delivery. The combinations often include at least one of the functionalization strategies already explored in the previous categories, such as transferrin, ApolipoproteinE and anti-melanotransferrin Ab.

Combination approaches not only facilitate targeting strategies but also enable techniques to increase accumulation by reducing clearance or improving biocompatibility. For instance, Kuo *et al* integrated their functionalization strategies (proteins and Abs targeting BBB-specific receptors) with tamoxifen. This small molecule has been reported for its capacity to block multidrug resistance-related proteins (Kuo et al., 2021), which are part of the metabolic activity involved in the expulsion of substances that cross the BBB. Similarly, the integration of peptides with biomimetic systems, such as macrophage- and neutrophil-coated nanoparticles, serves as another illustration of the potential for advanced combination strategies. This approach is exemplified in the studies by Han et al., 2021 and Chen et al., 2018.

On the contrary, the experimental design and results of Guo et al., 2020 suggest that adding transferrin to NLCs already functionalized with docosahexaenoic acid might not represent a significant additional enhancement in permeability for their particular formulation. Similarly, Han et al., 2021 observed no substantial improvements from the addition of triphenylphosphine cation (TPP) to macrophage coated SLNs already functionalized with rabies virus glycoprotein peptide. Ray et al., 2021 reported no additional improvements with the addition of penetrating peptides to LNPs already coated by CCR5 aptamer. These examples highlight the complexity and uncertainty of additive effects, indicating that multiple functionalization does not necessarily imply better permeability outcomes. However, other variables might be influencing the experimental results. For example, in the study from Guo et al., 2020, their particular results showed that that highest permeation enhancement seems to shift from double to single functionalization from day one to day three, as illustrated in Fig. 6.

Table 4
Comparative analysis of key study features for other functionalization strategies for lipid nanoparticles to overcome the BBB.

Study	Type of lipid nanoparticle	Particle size;Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
Banerjee et al. (2016)	SLNs	178 ± 9.4 nm; −17.4 ± 1.7 mV	Tyr-3-octreotide	Paclitaxel	***In vivo*** Sprague Dawley Rats	Functionalization led to an approximate **1.29**-fold increase in brain accumulation and around a **1.59**-fold rise in brain tumor accumulation. Brain accumulation was calculated using radioactivity measurements in tissue samples observed up to 6 h post-administration.	The authors' data provide a solid basis for discerning the effects of nanoparticle functionalization on organ-specific accumulation. The formulation showed significantly higher anti-tumor efficacy and improved survival rates, compared to un-functionalized nanoparticles, free paclitaxel and saline control, in that order.
Vijayakumar et al. (2016)	Liposomes	64.5 ± 5.56 nm; −1.05 ± 1.12 mV	TPGS[1]	Resveratrol	***In vivo*** Charles Foster rats	Encapsulation of resveratrol in plain liposomes increased brain concentration by 8.58-fold*, while encapsulation in functionalized liposomes increased it by 10.95 times* (functionalization only increased concentrations by about **1.27**-fold*). Brain concentration of resveratrol was measured with HPLC-UV on tissue samples of animals scarified 90 min after administration	In all organs except the brain, functionalization reduced resveratrol accumulation compared to plain liposomes. The authors also proved the biocompatibility of the formulation in haemolysis and platelet aggregation studies.
Ma et al. (2020)	Lipid nanoparticles[3]	About approximately 170 to 180 nm; About approximately 40 mV	Neurotransmitter –derived synthetic lipids(tryptamine)	i Amphotericin B ii Antisense oligonucleotides iii Genome editing fusion protein	***In vivo*** BALB/C mice (female)	Authors described that the incorporation of the neurotransmitter–derived synthetic lipids led to **brain accumulation** of a fluorescent dye in otherwise impermeable lipid nanoparticles. They compared the relative intensity of three different neurotransmitter–derived synthetic lipids and found brain accumulation only with the lipid modified with dimethyltryptamine.	The authors combined the neurotransmitter with lipid tails with different carbon lengths. They found that the relative intensity of brain accumulation was different for each lipid tail. The authors used their formulation to successfully deliver small-molecule drugs, nucleic acids and genome-–editing proteins to the brain after vein injection. Each formulation used a different base nanoparticle formulation but used the same neurotransmitter –-derived synthetic lipids.
Wu et al. (2023)	Lipid nanocapsules[4]	31.2 ± 0.8 nm; −5.2 ± 0.26 mV	Menthol	Verbascoside	***In vitro*** hBMECs	Compared to unfunctionalized nanoparticles, the incorporation of menthol increased the transportation ratio of the BBB model by 1.29-fold (at 0.1 μg/mL) and **2.20**-fold* (at 1 μg/mL). Compared to free drug the increase was up to 3.84-fold*.	The encapsulated therapy combining verbascoside with menthol decoration demonstrated a neuroprotective effect in a neurotoxic cell model. It effectively reduced reactive oxygen species, apoptotic mediators, and the aggregation of Aβ peptides, showing promising potential for research into protein tau phosphorylation in the treatment of Alzheimer's disease.
Zwain et al. (2023)	NLCs	gamma-linolenic acid, 157.36 ± 1.53 nm; −19.13 ± 0.20 mV alpha-linolenic	Gamma-linolenic acid and Alpha-linolenic acid	Docetaxel	***In vitro*** HBMEC, HBVP and HA	After 6 h of administration in the *in vitro* model, both functionalized nanoparticles showed a fluorescence rate **2**-	The authors also demonstrated enhanced permeability in a blood–brain tumor barrier model. Their

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Table 4 (*continued*)

Study	Type of lipid nanoparticle	Particle size;Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
		acid, 155 ± 0.10 nm; −16.03 ± 1.20 mV				fold higher than that of the unfunctionalized nanoparticles.	results showed increased absorption of the functionalized NLCs, which preferentially targeted glioblastoma cells over healthy cells, thereby amplifying the induced toxicity of docetaxel.
Patra et al. (2024)	SLN	140 ± 30 nm; −14 ± 5 mV	S-(−)-γ-amino-α-hydroxybutyric acid (GAHBA) derived lipids	Chlorambucil	***In vivo*** female C57BL/6 mice	The authors evaluated the biodistribution of radiolabeled SLNs and observed brain uptake. This evaluation did not provide a comparison with non-modified SLNs	Authors conducted a docking study to investigate the interactions between γ-aminobutyric acid (GABA) and S-(−)-γ-amino-α-hydroxybutyric acid (GAHBA) with the GABA receptor. Additionally, they evaluate the transfection efficiency on U87MG and human prostate cancer PC3 cell lines.
Wu et al. (2024)	Lipid nanoparticles[3]	78.7 ± 0.79 nm; 7.9 ± 1.24 mV	Borneol	Exenatide	***In vitro*** bEnd.3 ***In vivo*** male C57BL/6 mice	Flow cytometry results showed higher uptake of borneol-modified LNPs compared to plain LNPs, in SH-SY5Y cells in the abluminal chamber. *In vivo* results showed approximately a **2**-fold** increase in brain accumulation, measured by fluorescence intensity 4 h post-administration.	The authors propose that their borneol-modified LNPs enhance permeability by translocating tight junction proteins on bEnd.3 cells. The also assessed safety and biocompatibility of their LNPS, as well as its effects on motor symptoms relief, dopaminergic neuron restoration, and amyloid protein production in a Parkinson's disease mouse model.
McConnell et al. (2019)	Liposomes	57 ± 0.79 nm; Zeta potential was not reported	Transferrin receptor aptamer	Dopamine aptamer	***In vivo*** male CD1 mice	Fluorescence microscopy revealed enhanced brain delivery from transferrin aptamer functionalization, evidenced by distinct fluorescence distribution (not quantified).	Prior to this, the authors screened various aptamers to identify the most effective for liposome functionalization. Additionally, they conducted behavioral tests to assess the impact on neural dopamine levels and confirmed elevated concentration of neural dopamine, and yet demonstrating that chronic systemic administration of their formulation did not produce adverse neurobehavioral or neurodegenerative effects.

Notes:
Abbreviations: GAHBA: S-(−)-γ-amino-α-hydroxybutyric acid; HA: Human astrocytes; HBMECs: Human brain microvascular endothelial cells; HBVP: Human brain vascular pericytes; NLCs: Nanostructured lipid carriers; SLNs: Solid lipid nanoparticles; TPGS: D-α-Tocopherol polyethylene glycol 1000 succinate.
Reported statistically significant permeability improvements (p value: * < 0.05).
1- D-α-Tocopherol polyethylene glycol 1000 succinate is a PEGylated derivate of vitamin E.
2- The authors conducted their research using a mouse model with induced traumatic brain injury.
3- Not categorized, possibly SLNs.
4- Reverse micelles encapsulated into a lipid nanoparticle, possibly with an structure similar to NLCs.

Table 5
Comparative study of studies of lipid nanoparticles using mixed functionalization methods to overcome the BBB.

Study	Type of lipid nanoparticle	Particle size;Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
Basso et al. (2020)	Ultra small NLCs	124.0 ± 0.2 nm; 7.5 ± 0.4 mV	Hyaluronic acid conjugated with: i H7k(R2)2 peptide ii Folic acid	i Curcumin ii Atorvastatin	***In vivo*** Nude mice (male)	For atorvastatin, encapsulation alone led to a 9.32-fold increase in the brain's AUC. Double functionalization also resulted in higher AUC compared to the free drug (2.94-fold), but functionalization alone led to a decrease compared to plain NLCs (**0.315**-fold). As for curcumin, encapsulation alone reduced the brain AUC (0.33-fold) but double functionalization led to a 5.97-fold increase compared to the free drug and a **27.69**-fold compared to plain NLCs. Calculation was performed by quantification of curcumin and atorvastatin in tissue samples by HPLC-UV	The authors assessed organ-specific selectivity using the Drug Selectivity Index (DSI) and compared encapsulated versus non-encapsulated administration with the Drug Targeting Index (DTI), highlighting the formulations' efficacy in minimizing drug accumulation in organs like the liver and spleen. Additionally, evaluations of cell viability, uptake, and pathways, alongside anti-tumour activity in a mouse model, comparing functionalized nanoparticles with non-encapsulated drugs and saline controls, demonstrated that functionalized nanoparticles more effectively hindered glioblastoma progression and offered a safer profile
Li et al. (2016)	Liposomes	78.87 ± 2.29 nm; −15.8 ± 0.26 mV	Glutamate-TPGS[1]	Docetaxel	***In vivo*** Male Kunming mice	The modification with only TPGS, and TPGS combined with glutamate, enhanced the brain accumulation of liposomes encapsulating a fluorescence dye. However, this accumulation was not quantified.	The authors demonstrate the targeting efficiency to Large Amino Acid Transporter 1 (LAT1), present in the BBB and overexpressed in some types of cancer.
Kuo et al. (2022)	SLNs	About 200 nm; About −28 and −38 mV	Folic Acid and Transferrin targeting peptide (Tf)	i BV6 ii GDC0152[1]	***In vitro*** HBMECs, HAs and HBVPs	The inclusion of Tf on the surface of the SLNs increased the permeability of BV6 and GDC0152 by about **1.7**-fold* compared to unfunctionalized SLNs, and more than 2.5-fold** compared to free drugs. Permeability calculations were based on drug concentrations measured in the abluminal chamber over 4-hour period	Through immunofluorescence staining, flow cytometry, and Western blot analysis, the effectiveness of folic acid and transferrin-modified SLNs encapsulating BV6 and GDC01521 was demonstrated in the downregulation of apoptosis inhibitors (cIAP-1, XIAP), which act on caspase 3. This downregulation consequently led to an upregulation of caspase 3 content.
Guo et al. (2020)	NLCs[2]	93.02 ± 4.33 nm Between −30 and −35 mV	Docosahexaenoic acid (DHA) and Transferrin targeting peptide (Tf)	Darunavir	***In vitro*** hCMEC/d3 ***In vivo*** ICR mice (both sexes)	*In vitro* studies highlighted the significant role of DHA in enhancing cellular barrier penetration by nanoARVs, while the presence of Tf decoration did not notably affect this outcome. After 6 h, permeation compared to the free drug increased to 3.16** with 5 % DHA, 3.21** with 5 % DHA-Tf, 7.67** with 15 % DHA, and **8.99**** with 15 % DHA-Tf.	After comparing results from empty nanoparticles (both with and without functionalization), free drug, and functionalized drug-loaded nanoparticles, the authors observed enhanced anti-HIV activity in the latter, evaluated in a 293 T cell model expressing the p24 HIV capsid protein. The authors also demonstrated the stability and biocompatibility of their preparations.

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Table 5 (*continued*)

Study	Type of lipid nanoparticle	Particle size;Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
						In vivo studies indicated an improvement in darunavir brain accumulation attributable to both DHA and Tf over a 3-day evaluation period. On day one, the greatest enhancement compared to free drug was observed in nanocarriers including both DHA and Tf (**5.93**)*, and by day three, the highest increase was seen in only DHA-modified carriers (**4.41**)**. Refer to Fig. 6 for more details. Darunavir concentrations in the abluminal chamber and tissue samples were measured using liquid chromatography- mass spectrometry.	
Kuo and Hsu (2017)	SLNs	Between 70 and 190 nm Between −15 and 42 mV	Anti-melanotransferrin and ApolipoproteinE	Doxurrubicin	***In vitro*** HBMEC/HA	Compared to plain nanoparticles, the inclusion of anti-melanotransferrin increased the permeability of the doxurrubicin up to 1.5-fold. For the case of the double-functionalized nanoparticles, the increase was up to **4**-fold. Permeability calculations were based on drug concentrations measured in the abluminal chamber over 5-hour period	Antiproliferative assays on U87MG cells demonstrated enhanced efficiency due to functionalization. The highest efficacy was observed in doubly modified nanoparticles, followed by (in this order): anti-melanotransferrin, plain nanoparticles, and free drug, showing lesser effects.
Han et al. (2021)	SLNs (Macrophage membrane-coated)	123.19 ± 0.57 nm; 19.13 ± 0.33 mV	Macrophage membrane-coated; RVG and TPP peptides	Genistein	***In vitro*** bEnd.3, HT22 and astrocytes ***In vivo*** SD rats, C57BL/6J mice and APP/PS1 mice	Results from flow cytometry on HT22 cells after crossing the BBB model showed an increase of **11.56**-fold* for the macrophage membrane-coated SLNs with both peptides. Macrophage membrane-coated SLNs with RVG showed 10.63-fold*, the macrophage membrane-coated SLNs with TPP 3.85-fold), and the macrophage membrane-coated with no peptides 1.85-fold. All results obtained after 12 h of incubation and compared to free fluorescent dye. The *in vivo* results on SD rats showed an accumulation in brain tissue up to **8**-fold* for both the fully functionalized SLNs and the macrophage membrane-coated SLNs with RVG peptide. The macrophage membrane-coated SLNs with TPP showed about a **2**-fold increase, all compared to the macrophage membrane-coated SLNs with no peptides. Fluorescent signals from	The authors conducted preliminary safety tests on HT22 neurons and assessed how peptide incorporation reduced the ability of macrophage membrane-coated SLNs to evade the reticuloendothelial system and prolong circulation times. *In vitro* studies on HT22 neuronal cells demonstrated enhanced anti-apoptotic effects and reduced mitochondrial reactive oxygen species with the fully functionalized SLNs. *In vivo*, Morris water maze tests on APP/PS1 transgenic mice revealed improved behavioral outcomes for the fully functionalized SLNs, indicating potential as an effective treatment for slowing Alzheimer's disease progression. These studies also showed a reduction in neuroinflammation, and a decrease in markers of oxidative stress and Alzheimer's disease progression.

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Table 5 (*continued*)

Study	Type of lipid nanoparticle	Particle size;Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
						brain homogenates were analyzed to obtain the accumulation results.	
Chen et al. (2018)	SLNs (neutrophil-SLNs co-vehicle).	Between 30 and 60 nm; 12.6 mV	Neutrophil-coated PGP (N-Acetyl-Pro–Gly–Pro)	Baicalein	***In vivo*** Tissue distribution in rats	After 7 days of administration, the fluorescence intensity in the basolateral amygdala were **4.6** times higher for the neutrophil-coated SLNs decorated also with PGP, compared to the neutrophil co-vehicle without peptide.	The binding capacity and stability of PGP-SLNs to neutrophils demonstrated high affinity. The formulations exhibited *in vitro* antioxidative effects and enhanced baicalein efficacy due to encapsulation in SLNs. Functionalized NPs showed a greater antidepressant effect in rat behavioral models (reduced immobility and increased swimming times) than unfunctionalized SLNs, with both nanoparticle formulations outperforming the free drug in antidepressant efficacy.
Hernando et al. (2022)	NLCs	264.1 ± 16.0 nm; 20.5 ± 0.8 mV	Chitosan and Trans-activating transcriptional activator	Glial cell-derived neurotrophic factor, or Vascular endothelial growth factor	***In vitro*** hiPSC	Crossing of the double-functionalized NLCs was detectable by confocal microscopy, whereas the chitosan-modified alone was not detectable across the BBB model, after 2 h of incubation.	In a microglial cell line model with an inflammation inducer, the formulation demonstrated antioxidative stress properties and effectively reduced the inflammatory state.
Kuo and Cheng (2016)	SLNs	About approximately 105 to 180 nm; About approximately 17 to 38 mV	i Lactoferrin (Lf) ii Tamoxifen (TX)[1]	Carmustine	***In vitro*** HBMECs/ HAs cells	Lactoferrin and tamoxifen double-functionalized SLNs increased permeability coefficient by approximately **8.5**-fold* compared to unfunctionalized SLNs. Tamoxifen alone improved permeability by 4-fold. Results on SLNs modified only with lactoferrin were not reported. Permeability coefficient were calculated based on carmustine concentrations measured in the abluminal chamber over 5-hour period	Double-functionalized SLNs decreased viability of U87MG cells by almost 75 %. SLNs functionalized only with tamoxifen were the second most effective (approximately 50 %), followed by SLNs functionalized only with lactoferrin (approximately 30 %). Unfunctionalized SLNs had an effect comparable to the control.
Kuo and Wang (2017)	SLNs	About approximately 165 to 225 nm; About approximately 6 to 12.5 mV	i Lactoferrin ii Wheat germ agglutinin (WGA)	Etoposide	***In vitro*** HBMECs/ HAs cells	Compared with unfunctionalized SLNs, the permeability index of etoposide increased by 3-fold with the SLNs modified with only Lf. The inclusion of WGA (double functionalization) increased the permeability by **5.5-fold**. Permeability calculations were based on etoposide measurements in the abluminal chamber over a 5-hour period.	SLNs containing etoposide and co-functionalized with Lf and WGA reduced the viability of U87MG cells (mortality approximately 30 %). Modification with only Lf caused the mortality of approximately 25 % of cells, while unfunctionalized SLNs had an effect on about 20 %.
Kuo and Wang (2016)	SLNs	From approximately 150 to 280 nm; About approximately 12 to 27 mV	i Anti-Melanotransferrin (MA) ii Tamoxifen (TX)[1]	Etoposide (ETP)	***In vitro*** HBMEC-HA	Double functionalization (MA and tamoxifen) increased the etoposide permeability coefficient by almost **2**-fold* compared to functionalization with tamoxifen only. Controls did not include plain SLNs. Permeability calculations	Based on calcein-AM clearance studies, the authors showed that functionalization with melanotransferrin did not affect the tamoxifen capacity to decrease the activity of multidrug resistance-related proteins. The functionalized SLNs were effective in treating U87GM cancer cells,

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Table 5 (*continued*)

Study	Type of lipid nanoparticle	Particle size;Zeta potential	Functionalization	Drug	BBB model	Changes in NP or drug distribution	Other results
						were based on etoposide measurements in the abluminal chamber over a 5-hour period.	reducing their survival to approximately 25 %.
Ray et al. (2021)	Lipid nanoparticles[3]	Specific reported values range between: 54.2 ± and 69.2 ± nm; Between −11 ± and −2.9 ± mV	Aptamers: GP160 and CCR5 Peptides T7 and Tat	None	***In vitro*** hCMEC/D3	Authors found that CCR5 aptamer facilitated passage through the BBB model, whereas the gp160 aptamer did not. They also noted that incorporating cell-penetrating peptides, Tat and T7, did not enhance BBB penetration beyond that achieved with aptamer-loaded LNPs alone. In this study, evaluation were performed by seeding different type of target cells in the abluminal chamber, and evaluating fluorescent intensity after 24 h of incubation with the NPs.	The authors also found that functionalization with CCR5 aptamer also facilitates the uptuke of the LNPs into CCR5-expressing cells. Additionally, they demonstrate the low immunogenic and low toxic profiles of their formulation.

Notes:
Abbreviations: AUC: Area under the curve; BBB: Blood-brain barrier; Bend.3: Brain endothelial cells derived from mice; CCR5: RNA aptamer specific for the HIV-1 entry coreceptor C–C chemokine receptor type 5; DHA: Docosahexaenoic acid; DSI: Drug selectivity index; DTI: Drug Targeting Index; ETP: Etoposide; GP160: RNA aptamer specific for the HIV-1 envelope protein gp160; HA: Human astrocytes; HBMECs: Human brain microvascular endothelial cells; HBVP: Human brain vascular pericytes; HBMEC/D3: Human brain microvascular endothelial cells; hiPSC: Induced pluripotent stem cell; HIV: Human immunodeficiency virus; LAT1: Large amino acid transporter 1; LNPs: Lipid nanoparticles; MA: Anti-melanotransferrin; NLC: Nanostructured lipid carriers; PGP: N-Acetyl-Pro–Gly–Pro; RVG: rabies virus glycoprotein (peptide); SLNs: Solid lipid nanoparticles; TA: Tamoxifen; Tf: Transferrin targeting peptide; TPGS: D-α-Tocopherol polyethylene glycol 1000 succinate; TPP: triphenylphosphine cation; WGA: Wheat germ agglutinin.
Reported statistically significant permeability improvements (p value: * < 0.05; ** < 0.01).
1- Antagonists of apoptosis inhibitor proteins that can induce an apoptotic signaling pathway against tumor cells.
2- Authors identify their lipid nanocarriers as "NanoARV" because of their anti-retroviral application.
3- Not categorized, possibly corresponds to NLC.

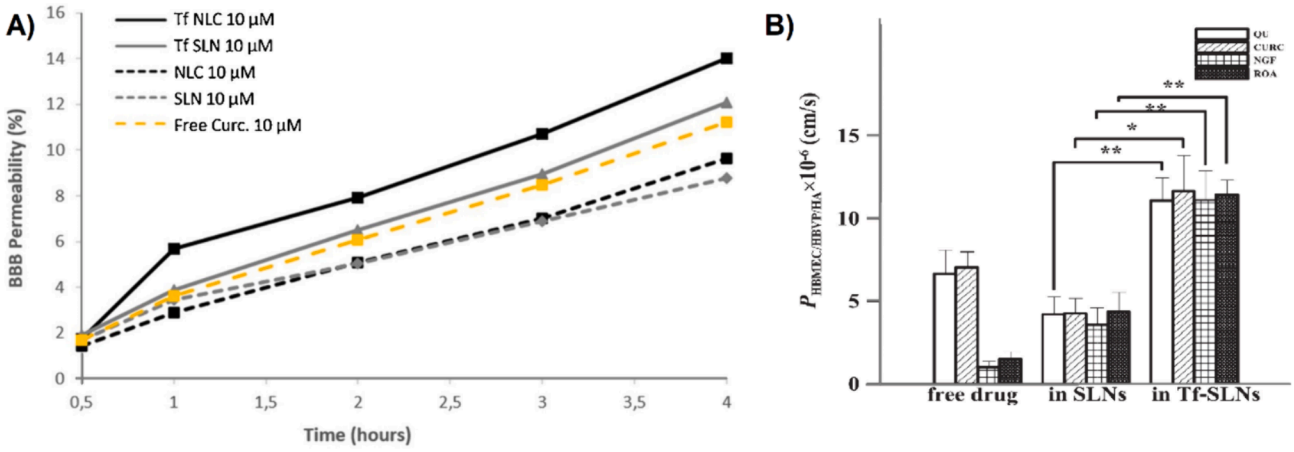

Fig. 4. Impact of nanoparticle encapsulation, peptide inclusion, and nanoparticle and drug nature in permeability across BBB cellular models. A) Curcumin encapsulation in SLNs and NLCs led to reduced BBB permeability, but the Inclusion of transferrin peptide resulted in increased permeability, especially for NLCs. Permeability for modified SLNs and free drug (Curc) were comparable. B) Encapsulation in SLNs reduced the permeability of free quercetin (QU) and curcumin (CURC), but increased the permeability of nerve growth factor (NGF) and Rosmarinic acid (ROA), bringing all four drugs to comparable levels. Further transferrin inclusion significantly increased the permeability of all four drugs; *p value < 0.05, **p value < 0.05. Reprinted with permission from Neves et al., 2021 and Kuo et al., 2020.

Regarding the employed evaluation methods, 5 out of 12 studies conducted *in vivo* assessments, with two integrating *in vitro* analyses as well (Han et al., 2021; Guo et al., 2020). In the study from Guo et al., 2020, the conclusions drawn from both *in vitro* and *in vivo* models regarding the significance of the second functionalization (addition of transferrin peptide) were similar. However, in the study from Han et al., 2021, the interpretation regarding the impact of adding one versus two peptides to the macrophage membrane-coated SLNs varied depending on whether *in vitro* or *in vivo* results were considered. Basso et al., 2020 present another instance of the divergent correlation between *in vitro*

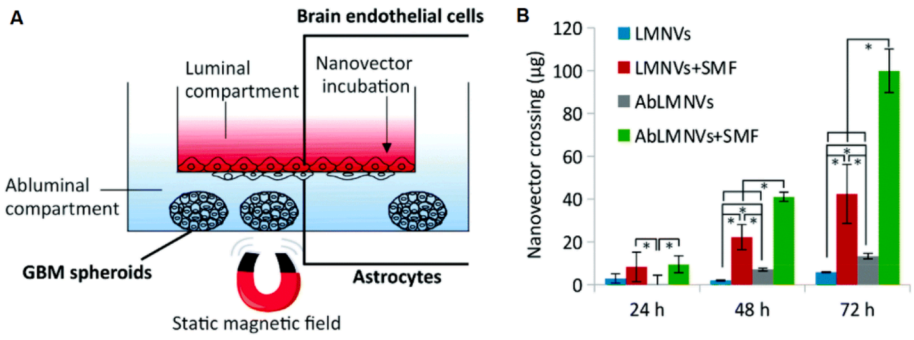

Fig. 5. Synergistic effect of anti-melanotransferrin antibody functionalization on supramagnetic nanoparticles. A) Diagram of the cellular model including magnetic stimulation that forced the NPs to cross the cellular layer, and B) Nanoparticle crossing predominantly affected by magnetic stimulation. LMNVs = lipid magnetic nanovectors; SMF=static magnetic field; Ab = antibody- modified nanovector. * p value < 0.05. Figure adapted from (Muntoni et al., 2019) with permission from the Royal Society of Chemistry ©.

and *in vivo* findings. Despite relying solely on an *in vivo* model for BBB permeation, their study assessed anti-tumor efficacy both *in vivo* and *in vitro*. The research revealed weaker *in vitro* anti-tumor activity compared to *in vivo* results. Their study also exploited *in vivo* evaluations to investigate functionalization effects on organ accumulation. Notably, developing two indicators: the Drug Selectivity Index (DSI) and Drug Targeting Index (DTI), offering valuable insights into organ-specific selectivity evaluations (Basso et al., 2020). The evaluation included two different drugs, and the permeability, permeability enhancement, DSI and DTI values were different for each drug.

As previously stated, multiple functionalization did not necessarily imply better permeability. However, it is noteworthy that some of the more pronounced differences in permeabilization can be found within this category. For instance, Basso et al., 2020 found a 27.69-fold increase in *in vivo* brain concentrations for curcumin. Han et al. (2021) observed an 8-fold increase. Additionally, studies by Kuo *et al* in the protein (Kuo et al., 2021) and antibodies categories (Kuo and Lee, 2016) show that double-functionalized NPs may have significantly higher permeability than single-functionalized or plain NPs.

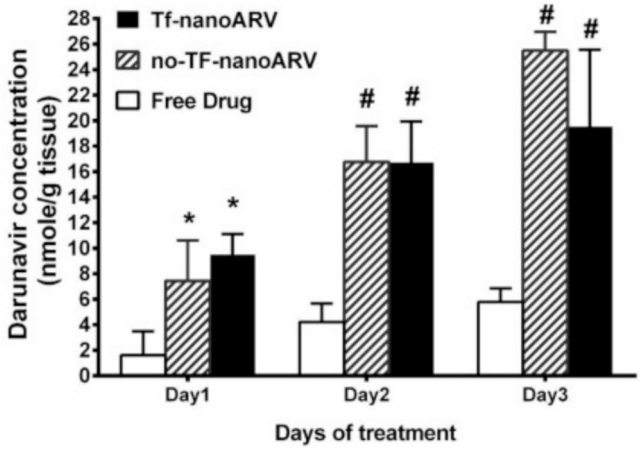

Fig. 6. Temporal impact of single and double functionalization on darunavir brain concentrations. The reported results show darunavir *in vivo* brain concentrations after the administration of the free drug, the drug encapsulated in nanoARV (NLCs functionalized with docosahexaenoic acid), and the nanoARV with additional transferrin functionalization. The more pronounced effect of one nanosystem over the other depends on the day of evaluation; * p value < 0.05 vs free drug; # p value < 0.01 vs free drug. Reprinted with permission from Guo et al., 2020.

3.6. Non-modified LNPs, or modified with regularly used materials

As outlined earlier, NPs can cross the BBB even without specialized functionalization (Neves et al., 2021; Arduino et al., 2020; Pucci et al., 2020). This observation is supported by results from earlier sections, where the impact of functionalization was often evaluated in comparison to the permeability of unmodified LNPs. Consequently, in our systematic review, we also found studies that reported on LNPs formulations without any functionalization methods (Khan et al., 2020; Liu et al., 2022; Kumar et al., 2016; Gadgil et al., 2018; Ghasemian et al., 2017; Nasir et al., 2023; Rubab et al., 2021; Arduino et al., 2020; Zwain et al., 2021; Chen et al., 2017; Rojekar et al., 2021; Huang et al., 2020; Gomes et al., 2016; Makhdoomi et al., 2022; Mante et al., 2021; He et al., 2019; Ebrahimi et al., 2022; Marinelli et al., 2023; Pandian et al., 2021) and yet showed promising results for brain delivery. Additionally, we found research focusing on formulations that primarily utilize surfactants (Pavlov et al., 2020; Lahkar and Kumar, 2018; Graverini et al., 2018; Esposito et al., 2017; Meng et al., 2016) and PEGylation (Buzyurova et al., 2020; Vijayakumar et al., 2016) as their chosen strategies for functionalization. However, since the addition of surfactants and PEGylation are regularly adopted strategies in the manufacturing of different types of LNPs, we categorize these kind of functionalization strategies into the same category as non-modified or modified with regularly used materials, for the context of this review.

Table S1 and Figure S1 provide more details on key features of the studies included in this passive targeting category. These results illustrate that even these regular modifications can significantly improve brain drug distribution. Moreover, some studies evaluate and find positive effects on therapeutic efficacy and safety profiles. For example, studies showed therapeutical potential in anxiolytic (Khan et al., 2020; Rubab et al., 2021), antitumor (He et al., 2019; Nasir et al., 2023), and antioxidant (Marinelli et al., 2023; Kumar et al., 2016) activity evaluations, as well as positive results in seizure (Mante et al., 2021), cognitive impairment (Makhdoomi et al., 2022), and oxidative damage (Huang et al., 2020) models. In accordance with previous observations, NLCs showed higher BBB penetration compared to SLNs when those alternatives were evaluated in the same study (Kumar et al., 2016; Ghasemian et al., 2017).

4. Cross-category evaluation of permeation enhancement

As stated in Section 2, the results from 32 studies were collected for data analysis. These studies provided quantification data that allowed determination of the extent to which permeability increased due to functionalization effects. Due to comparisons of different combinations or combinations of *in vivo* and *in vitro* results in some studies, these 42 studies yielded 91 different ratios for data analysis. Fig. 7 shows the data distribution and variable impacts observed.

To further elucidate these findings, we conducted a principal component analysis (PCA) with a covariance matrix, detailed in the Supplementary Information (Figures S2 to S4). Insights from the first two components, explaining 98.1 % of the data variability, allowed identification of a significant impact on BBB permeability for particle size, type of lipid nanoparticle and category of functionalization. Conversely, the PCA revealed that zeta potential does not significantly contribute to explaining data variability for the included studies. This suggests its lesser role in the predictive modeling of permeability enhancement. Subsequent classification through CART® analysis (Supplementary Figures S5 and S6) indicated that particle size was the predominant predictor for this particular set of observations. This was

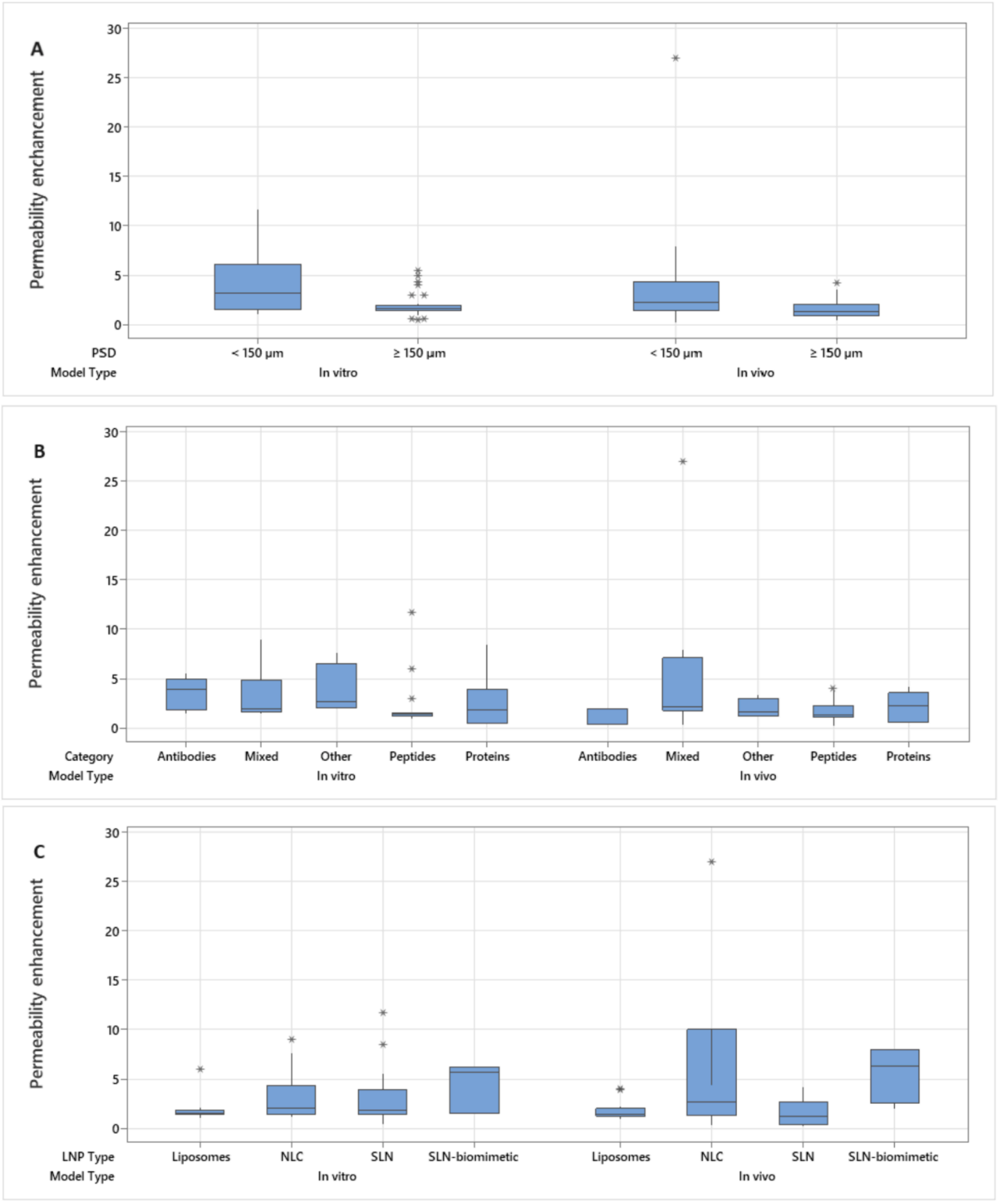

Fig. 7. Cross-category impact on permeation enhancement across the BBB. Analysis by particle size (A), functionalization category (B) and the type of lipid nanoparticles (C) from 42 studies that allowed permeability enhancement. Variable significance is supported through principal component analysis and CART® classification, complemented by main effects and variance analyses detailed in Supplementary Information (Figures S2-S8).

followed by the functionalization category and the type of lipid nanoparticle. Figure S7 and the corresponding analysis of variance validated the categorization of particle sizes based on different response patterns in permeation enhancement ratios. Moreover, the main effects analysis shown in Figure S8 confirmed the significant effects of particle size, functionalization category and the type of lipid nanoparticle on the evaluated permeation enhancement ratios.

5. Discussion

Across all categories, the functionalization of LNPs consistently improved their ability to cross the BBB. This enhancement improved drug delivery to the brain while proving their biocompatibility. These strategies emerge as potential tools to overcome the restrictive properties of the BBB. The variety of therapeutic agents applied in the observed studies highlights the adaptability of LNPs as versatile carriers. Regarding the encapsulated drugs, our findings reveal a predominance of natural extracts and small molecules, with a surprisingly limited application to nucleic acid brain delivery. However, examples such as the protein expression observed in the studies by Arora and Singh, 2021; Arora et al., 2020 and Ma et al., 2020, or the encapsulation of siRNA and mRNA by Tang et al., 2024 and Bi et al., 2023 underscore the high potential of this delivery systems in gene and RNA therapy.

This limited use within the included studies contrasts with other development areas of nucleic acid delivery, where LNPs are at the forefront of clinical innovation. This might be due to a relatively unexplored potential for nanoparticle brain delivery and active targeting. This predominance of therapeutic evaluations represents an opportunity for innovative research in gene therapies and nucleic acid approaches to brain drug delivery. Despite these observations, the results also showed versatility in terms of therapeutic objectives, from chemotherapy agents to anti-inflammatory drugs.

To interpret the permeability enhancement ratios derived from various studies, it is important to consider that each ratio has its own confidence intervals. These intervals arise from the inherent data uncertainty of each reported result. Such intervals suggest caution in interpreting minor differences, as they may not be statistically significant. In fact, only a portion of the studies included in this review explicitly report the statistical significance of their permeability comparisons, as detailed in Tables 1–5 and S1. Regardless, a comprehensive analysis of collective data trends across studies provides a robust foundation for understanding the overall impact of LNPs functionalization on BBB permeability. This analysis algo helps in identifying meaningful enhancements in drug delivery efficiency through LNPs.

Fig. 7 (and Figures S2-S8) shows particle size, functionalization category and type of LNPs as the main descriptors of patterns in permeability enhancement across the studies included in this review. By identifying the relative importance of these three factors, our analysis suggests that the formulation and design of the base nanoparticle (size and composition) are as important as the functionalization studies.

Regarding the first parameter (particle size), our findings show that LNPs smaller than 150 nm exhibit greater potential for permeation enhancement (Fig. 7-A). This aligns with the generally accepted claim that smaller particles are more capable of crossing biological barriers (Mishra et al., 2023; Correia et al., 2022; Jia et al., 2020). This may be due to the physical restrictions of larger particles and the higher surface area to volume ratio of smaller particles, allowing for closer interactions. In addition, our findings suggest that smaller particles are also more sensitive to permeability enhancement due to functionalization. The observed results lead us to define a size limit of 150 nm as “smaller nanoparticles” for the data included. Optimizing particle size is crucial for effective drug delivery systems targeting the CNS and reinforces the importance of meticulous nanoparticle design. This emphasizes the need for precision in engineering lipid nanoparticle systems to maximize their brain delivery capabilities.

The second variable aligns with the main objective of this review. Indeed, the cross-category results indicate that the selection of different functionalization strategies might affect the outcome in achieving brain transportation. However, the interpretation requires careful consideration of the type of BBB model applied. For example, the efficacy of antibodies and other strategies drops when comparing *in vitro* and *in vivo* results. Contrary, peptides, proteins and mixed strategies showed higher ratios for the studies reporting *in vivo* data (Fig. 7-B). The results for mixed functionalization strategies stand out due to their higher permeation enhancement values. This suggests that synergistic effects between different functionalization agents can lead to more efficient BBB penetration. While this synergy probably combines multiple mechanisms of transportation to optimize brain delivery, it might be associated with increased complexity and cost implications. Conversely, a closer examination of the data from Table 5 reveals that the highest permeability is not always achieved with dual-functionalized nanoparticles. For example, in the study from Han et al., 2021, the inclusion of TPP to RVG functionalization did not significantly enhance permeability beyond that achieved with the comparison to RVG alone. Similarly, in the study from Guo et al., 2020, the further addition of transferrin to DHA functionalization did not significantly impact permeability. This was especially the case in the presence of 5 % DHA. Such observations suggest that while the concept of combining functionalization agents is promising, synergistic benefits are unpredictable and do not always materialize. This can potentially complicate the design and increasing costs without commensurate gains in performance. This observation calls for a judicious balance between the complexity of functionalization strategies and their practical benefits.

The third variable with more relevance in describing the collected data is the type of nanoparticle (Fig. 7-C). One of the clearest and most consistent observations is the influence of the composition of the LNPs on both the base permeation and in the permeation enhancement. Notably, differences due to the nature of the lipid nanoparticle (NLCs or SLNs) were evident in every study included in our review. This was observed where both types were evaluated applying the same functionalization strategy, and evaluation model (presumably, under the most comparable conditions possible). Consistently, NLCs showed higher based permeability and greater permeation enhancement after functionalization (Neves et al., 2021; Pinheiro et al., 2020; Pinheiro et al., 2020; Kumar et al., 2016; Ghasemian et al., 2017). Other studies also reported results suggesting that lipid selection and quantity play a key role in determining BBB permeation (Reginald-Opara et al., 2022; Kuo and Lee, 2016; Gandomi et al., 2017; Kuo and Chao, 2016). This might be due to changes in the physicochemical properties of the LNPs. These changes affect the affinity and fusogenic properties when interacting to ECs in the BBB. The other category with higher permeation enhancement results is the biomimetic systems, which mimics blood cells. Their similarity to endogenous entities might explain their higher permeability, probably due to different mechanisms that other types of LNPs. This should carefully consider when selecting and applying functionalization strategies.

The impact of variables on the *in vivo* results in Fig. 7 appears clearer and showed more significance. In contrast, the differences on the *in vitro* results for functionalization category and type of lipid nanoparticles are less pronounced between categories. Thus, conclusions might be more affected by the confidence intervals above-mentioned. Additionally, the comparability of the results could be restricted by the remarkable heterogeneity observed. The reviewed studies collectively exposed several factors with an apparent influence beyond mere functionalization. Our analysis revealed substantial heterogeneity in experimental approaches and selected variables across studies, complicating direct comparisons. This also introduces added complexity to the analysis and design of LNPs systems for brain delivery. To address this challenge, we categorize the sources of variability into two predominant factors: nanoparticle composition and evaluation methodologies. Each sub-section aims to highlight the implications of these variables on the performance and assessment of LNPs from the observations drawn from different studies.

5.1. Nanoparticle composition variables as a source of variability

Differences due to the type of LNPs could be partially attributed to variations in NPs composition. Additionally, other changes related to composition could affect comparability. One example was found in the studies that included the evaluation of different cargoes using the same formulations. Their results showed that the drug itself also affects the effect of encapsulation and functionalization on BBB permeability. For example, the study from Basso et al., 2020 showed particularly different effects for the encapsulation and the functionalization of Atorvastatin and Curcumin, also affected by the comparison point (see Section 5.2.2 for more details). Kuo et al., 2021 found that their functionalization strategy improved the permeability of epigallocatechin gallate, but reduced it for resveratrol, and (Kuo et al., 2020) also observed differences according to the drug base permeability (Fig. 4-B). Collectively, these results strongly suggest that the effect from one drug cannot be extrapolated to other drugs. The relative impact of a given functionalization strategy should be assessed based on the same formulation and the same drug.

The quantity of functionalization moiety incorporated into LNPs is a significant variable that requires attention. Not every study or formulation explicitly indicates the rationale behind the selection of quantities. Nor do they include screening varying quantities of moieties and their effects. However, it could affect the functionalization outcomes. One example is the research of Dal Magro et al., 2018, where they showed that increases in functionalization content do not necessarily lead to direct linear enhancement in BBB permeability. In fact, the most significant permeability in their study was observed at the lowest ApoE4 levels. On the contrary, they found a direct relationship between rising ApoE4 levels and increased accumulation in peripheral organs. This underscores considerations for off-target effects (refer to Section 5.2.1).

Contrastingly, the study from Kuo and Lee, 2016 showed a direct positive correlation between increasing quantities of anti-aprotinin and permeability enhancement. These contrasting findings underscore the necessity of meticulously calibrating the amount of functionalization agents, highlighting that the efficacy of functionalization strategies is not solely dependent on quantity. Optimizing these quantities through comprehensive experimentation is crucial. This reveals that a higher quantity of functionalization moiety does not universally translate to improved outcomes. Additionally, results like the reported from Neves et al., 2021 raise questions about the possibility that functionalization might interfere with the stability of the NPs. This underscore the importance of performing stability studies, as indeed applied for several authors (e.g. studies: (Neves et al., 2021; Zhao et al., 2018; Singh et al., 2016; Chen et al., 2018; Guo et al., 2020; Nasir et al., 2023; Lahkar and Kumar, 2018).

Another relevant variable on nanoparticle composition is the inclusion of surfactants and PEGylation, which may explain the relatively high number of studies classified under Section 3.6. Their role could be explained by their impact on enhancing biocompatibility and increasing circulation times (Liu et al., 2022; Ghasemian et al., 2017; Buzyurova et al., 2020; Vijayakumar et al., 2016). However, it remains unknown whether this increase in permeability is due to a direct interaction of the PEG with the endothelial cells. Alternatively, the increase in circulation times results in a greater number of nanoparticles in contact with the BBB. Nevertheless, it is important to note that alongside prolonged circulation times, some authors argue that PEGylation can limit the ability of nanoparticles to effectively penetrate target cells (Zhao et al., 2018). Sometimes surfactants are also used to obtain “stealth” and “long-circulating” nanoparticles, protected from interaction with plasma components and increasing drug permeability by fluidizing cell membranes (Graverini et al., 2018). However, some authors claim that the permeability increases due to surfactants could be attributed to a mechanism different from that observed for peptide-functionalized prototype NPs (Kadari et al., 2018) or other moieties. Given that these commonly used materials can impact permeability, potentially through mechanisms different from those of the intended functionalization, careful consideration should be given to formula design. It is crucial to account for these components and optimize them before adding functionalization or integrate them as part of the experimental process.

The relevance of nanoparticle composition highlights the need for comprehensive investigations to enhance our understanding of how to optimize NPs for effective brain delivery. Given that the performance of NPs are highly dependent on parameter interactions (Vargas et al., 2023), adopting a Quality by Design (QbD) approach –as recommended by the International Conference on Harmonization’s Pharmaceutical Development Q8 guideline– offers a strategic pathway to address these complexities systematically. Improving the understanding of NPs interactions between functionalization and formulation can potentially increase the reproducibility, scalability, and stability of modified LNPs (Vargas et al., 2023; ICH, 2009). Future research directions could considerably benefit from the integration of QbD principles. This should focus on a comprehensive evaluation of base formulations before the addition of surface modifications, to facilitate a more targeted and effective exploration of nanoparticle-based CNS drug delivery systems.

5.2. Evaluation strategy variables as a source of variability

A critical aspect of our analysis involved the assessment of evaluation strategies used across various studies. Certainly, there are differences in the choice of BBB models, control strategies, and experimental setups such as doses, timing of analysis, quantification techniques, and efficacy evaluations. These methodological variations can substantially influence study conclusions. Such variations reinforce the difficulties in drawing direct comparisons between different studies and extracting general conclusions.

5.2.1. Type of evaluation model as a source of variability

Differences between *in vitro* an *in vivo* results are shown in Fig. 7. It also shows the limited correlation observed in the studies that combined both types of models, underscoring the need for cautious selection of evaluation strategies. Interestingly, the predominance of each type of strategy varies depending on the functionalization category, as observed in Fig. 3. For example, *in vivo* models are more commonly employed in the unfunctionalized category, as compared to the others. This observation could be attributed to a more extended understanding on the composition and behavior in biological *in vitro* systems for unfunctionalized systems. In contrast, the introduction of functionalization moieties into nanoparticles introduces new variables that may not have as much *in vitro* background. Consequently, this lack of extensive experience with functionalized nanoparticles could necessitate a more gradual transition to *in vivo* studies. This would help in understanding their complex interactions before moving to living organisms.

In vitro modeling, especially with human cell-based systems like the widely used hCMEC/D3 model, plays a key role in initial therapeutic assessments. It offers insights into potential BBB crossing. This cellular model is reported to have high representativeness (Pinheiro et al., 2020). However, some authors claim that the lack of shear conditions may lead to a higher permeability, giving rise to an overestimation of drug or nanoparticles crossing the cellular layer (Pucci et al., 2020). In fact, Pucci et al., 2020 developed a unique *in vitro* model including hCMEC/D3 and human astrocytes under dynamic flow conditions. They claimed that flow conditions were crucial to achieving more physiological phenotypic conditions for the barrier model, and thus a more accurate estimation of passage capacity.

The wide range of available *in vitro* methods differs in terms of complexity, reliability, cost and ease of implementation (Jagtiani et al., 2022; Morofuji and Nakagawa, 2020; Wilhelm and Krizbai, 2014). For instance, parallel artificial membrane permeability assay (PAMPA) based methods are fast and suitable for high throughput screening. However, their predictive value is questionable as it is limited to the prediction of passive diffusion (Appelt-Menzel et al., 2020; Müller et al.,

2015). Cellular-based models offer insights for both passive and active transport. However, they are labor-intensive, time consuming and generally low–throughput (Müller et al., 2015). Moreover, the physiological accuracy of these models depends on the cell source, culture complexity (ranging from mono culture to multicellular co-cultures) (Shah and Dong, 2022). It also depends on variations in methodological practices between different laboratories (Helms et al., 2015). Such variability suggests caution when comparing studies as well as selecting models for evaluating specific strategies.

In vivo studies could offer stronger conclusions on brain accumulation and efficacy, mainly due to their ability to provide comprehensive insights into systemic distribution, clearance, and overall drug delivery efficiency. This could be particularly relevant in active targeting of lipid nanoparticles to tissues other than the liver. This is due to the intrinsic hepatic accumulation of this type of vehicles. However, ethical, economic and logistical constraints often limit their application, making *in vitro* analyses a critical step for preliminary screenings. These *in vitro* results typically guide the progression to more costly and complex *in vivo* trials, underscoring the need for a judicious balance between the two types of models.

The relevance *of in vivo* models increases in terms of evaluating off-target accumulation. Detailed examinations of changes in biodistribution are essential to study not only brain accumulation, but also assessing organ-specific selectivity. These evaluations could potentially minimize off target effects, thereby enhancing the therapeutic profile of the formulations under study. It might also help to understand whether unintended off-target accumulation might contribute to lower brain accumulation. Additionally, it can reveal if positive results achieved by a particular functionalization strategy are due to extended circulation times, lower affinity for non-target organs, or the direct exploitation of the BBB's active transport mechanisms. The importance of this aspect could be illustrated by several studies included in this review that assessed the selectiveness of their NPs accumulation; including works where selectivity indexes were calculated (for example studies (Kuo et al., 2021; Gomes et al., 2016). Such methodological approaches offer potential contributions to the study and understanding of NPs brain accumulation mechanisms.

However, drugs developed and tested in animal models often exhibit different efficacy and toxicity profiles when tested in humans (Mehta et al., 2024; Zhang et al., 2023). The challenges in preclinical to clinical translation depend on variations in BBB characteristics across species (Syvänen et al., 2009; Verscheijden et al., 2021; Dao et al., 2024; Mármol et al., 2023). For instance, human brains feature a higher proportion and increased complexity of neocortical astrocytes compared to rodents (O'Brown et al., 2018). Additionally, they show less expression of claudin-5, a relevant protein for tight junction functionality (Mármol et al., 2023). Moreover, it has been demonstrated that differences in the sequences, expression and morphology of native transporter systems affect the transportation and distribution of substances to the brain (Zhang et al., 2023; Dao et al., 2024; O'Brown et al., 2018; Plotkin et al., 2000). For example, P-glycoprotein expression is reported to be lower in the human BBB compared to rodent BBB (Syvänen et al., 2009; Verscheijden et al., 2021). This limited correlation of preclinical models highlights the necessity for innovative approaches that more accurately mimic human BBB physiology and better predict clinical outcomes.

Emerging technologies like Organ-On-a-Chip systems (OoC) (Mármol et al., 2023; Vargas et al., 2021; Kang et al., 2021) offer promising avenues to bridge this gap. These systems simulate more accurately the biological and mechanical environment of human tissues, including the BBB. Despite their potential, our review found no studies within the scope of our review utilizing OoC models for BBB research, marking a notable area for future exploration. This absence underscores the ongoing need for innovative approaches. These approaches aim to increase IVIVC for brain delivery applications. Another area that could improve prediction capacity is exploiting the potential of advanced modeling techniques, such as computational simulations, to predict nanoparticle behavior and optimize delivery strategies. Until then, *in vivo* studies remain the basis for robust preclinical conclusions, allowing the evaluation of direct therapeutic outcomes in a more representative context, as well as assessment of biodistribution and organ interactions.

5.2.2. Selection of control strategy

Beyond model selection, the choice of an appropriate control strategy is probably one of the most relevant aspects. This choice is crucial to confirm the effect of a specific functionalization. However, we observed considerable heterogeneity in this regard. This ranged from the absence of a comparison, to the use of free drug, un-functionalized nanoparticles, or even partially functionalized (for dual strategy modifications). The lack of comparison with free drug (when possible) and with non-modified LNPs could lead to a false positive in the results. These false positives could be mistakenly attributed to the functionalization itself. For example, in the studies by Gandomi et al., 2017 and Muntoni et al., 2019, fully functionalized NPs showed greater permeability than free drug. However, it is noteworthy that functionalization reduce it compared to plain NPs. The results obtained by Loureiro et al., 2017 highlight the need for careful interpretation of positive outcomes. In their *in vitro* evaluation, they used an antibody with no specific target to the BBB as a control, yet it increased nanoparticle permeability compared to un-functionalized nanoparticles. Another illustration of the importance of establishing control strategies is the study by Basso et al., 2020, where functionalization successfully increased brain permeability compared to plain NPs, but had a negative effect compared to free drug, as the drug's permeability decreased when encapsulated.

The inclusion of different moieties as functionalization brings more relevance to the establishment of controls. The different studies presented in section 3.5 introduced extra complexity to comparison methodologies. This resulted in diverse approaches to calculating permeation enhancement. Not all studies utilized uniform controls or benchmarks. For instance, some compared the permeability of double-functionalized LNPs solely to plain LNPs (Chen et al., 2018; Basso et al., 2020), while others compared double-functionalized LNPs to those functionalized with a single moiety (Kuo et al., 2022; Guo et al., 2020; Hernando et al., 2022; Kuo and Wang, 2016). Several studies conducted more comprehensive evaluations, comparing plain, single-functionalized, and double-functionalized LNPs (Li et al., 2016; Kuo and Hsu, 2017; Kuo and Cheng, 2016; Kuo and Wang, 2017), with one study specifically assessing the separate effects of each single functionalization (Han et al., 2021). The potential conclusions that can be drawn vary with the complexity of the evaluation strategy. This subtly suggesting the potential layers of analysis involved. Particularly, the study from Han et al., 2021 allows differentiation of the individual effect of each peptide separately. Their research evaluating different functionalizations made in the same formulation, same composition and same model, one of the most comparable opportunities among the consulted bibliography.

The studies by Dal Magro et al., 2017 and Muntoni et al., 2019 highlight a crucial aspect in the design of control NPs. They modified their control NPs to avoid unintended biodistribution effects. By neutralizing reactive groups in the surface of un-modified NPs, they ensured that the only significant difference between the control and experimental nanoparticles was the absence of the functionalization effect. Such level of consideration when selecting a control strategy is crucial for accurately assessing the impact of functionalization, emphasizing the relevance of control conditions.

It is remarkable that in many cases the functionalization might be just one of many variables affecting permeability modulation. Adequate control selection might increase the strength of the conclusions. Otherwise, the reason for the permeability enhancement results cannot be fully understood, making it difficult to attribute permeability changes to the particle itself (and its composition) or to the specific modification strategy.

5.2.3. Other experimental considerations

Other experimental considerations can also affect the results of permeability. These factors can affect both specific conclusions and comparative analyses. Some examples observed within the publications included in this review involved the time of analysis. They also raise questions regarding doses or quantification techniques, and efficacy evaluations. For example, the perceived efficacy of certain strategies may shift based on the evaluation timeframe. This is exemplified by Guo et al., 2020, where the impact of transferrin inclusion varied between the second and third days of treatment (refer to Fig. 6). Similarly, Nasir et al., 2023 demonstrated that the effectiveness of functionalization decreased over time, from 2 to 6 h post-treatment (see Table S1). These results raise questions about the time dependency of effects on brain accumulation. This introduces more variables to consider when establishing appropriate evaluation periods for a given strategy.

Dosing methodologies might constitute another significant obstacle in standardizing evaluations across brain delivery studies. The variability in dosing details—ranging from precise dosage specifications to unclear or unreported dosages—complicates the direct comparison of results. It also confuses the estimation of optimal dosing strategies when designing a new study. This could be particularly crucial in discerning whether observed enhancements in brain permeability are due to the unique properties of the nanoparticle system. Alternatively, they might result from optimal dosing. Additionally, the possibility of receptor saturation (Pinheiro et al., 2020; Loureiro et al., 2015; Zhao et al., 2018; Dal Magro et al., 2018) increases the relevance for meticulous dosing methodologies to differentiate between formulation properties and dosing effects.

The application of different quantification methods also represents a limitation for comparison between studies. The distinction between detecting a labeling agent like a dye or radiolabel and directly measuring nanoparticles or therapeutic agents is significant. Depending on the observation, it might raise questions about whether nanoparticles are merely extending the drug's systemic half-life. Alternatively, they might be actively facilitating its BBB transport. The evaluation of therapeutic effects using *in vivo* models offers the opportunity for assessments in a context closer to clinical reality. However, the focus on cancer therapies within the reviewed literature brings to light the predominant use of cytotoxicity as a measure of efficacy. This requires careful consideration due to the inherent cytotoxic potential of nanoparticles themselves (Ilić et al., 2023; Nikzamir et al., 2021), emphasizing the importance of incorporating control evaluations for a comprehensive understanding of therapeutic efficacy.

Despite the aforementioned heterogeneity, it is important to acknowledge the considerable value of the studies included in this review. While we have highlighted certain differences that challenge our goal of making direct comparisons, this does not diminish the significance of their contributions to the field of nanoparticle functionalization. On the contrary, these studies collectively underscore the potential of functionalization strategies to overcome the BBB barrier, offering invaluable insights into diverse effects that significantly advance our understanding in this area. Each study, with its unique findings and approaches, contributes to this field of study, paving the way for future breakthroughs in CNS drug delivery.

6. Conclusions

This review systematically explored the diverse strategies employed in the functionalization of LNPs to enhance their BBB permeability, highlighting innovative approaches that show promise in facilitating brain delivery. Our findings offer comparative insights and might help to identify future research directions in the development of more effective LNPs systems for frug delivery to the brain

Across the evaluated categories, functionalization consistently shows potential to improve the delivery of therapeutic agents to the brain, demonstrating its role in active targeting through the BBB. Remarkably, the included data may indicate that studies utilizing NLC, those with particle sizes below 150 nm, or those employing mixed functionalization strategies, show superior permeation enhancement. This is relative to their unfunctionalized counterparts. However, it is clear that LNPs development is a complex and unique process for each formulation, where composition plays a pivotal role in dictating permeability. The development of a successful delivery strategy goes beyond mere functionalization. It requires prior optimization of the base nanoparticles, including their composition and physical properties. This can be achieved through the integration of Quality-by-Design principles.

Challenges within the field extend to understanding off-target accumulation and the complexities of correlating *in vitro* and *in vivo* studies. Our review also highlights the complexity and heterogeneity in evaluation approaches and the selection of control strategies. The variation in methodologies across studies poses a significant challenge for direct comparison and validation of results. Moreover, we identify the selection of appropriate control strategies as a critical factor. This significantly influences the interpretation of functionalization impact. These methodological considerations highlight the importance of adopting a holistic and standardized approach to research on brain delivery of LNPs, ensuring that findings are robust, comparable, reproducible, and translatable to clinical applications. Addressing these challenges could facilitate opening the gates to the full potential of LNPs in treating a broad spectrum of neurological and psychiatric disorders and brain tumors and revolutionize CNS disease management.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, authors used ChatGPT® in order to improve language quality and increase quality and readability of initial drafts. The tool assisted with refining complex sentence structures, providing synonymous expressions, and synthesizing lengthy descriptions to improve overall readability from sentences previously developed by the authors. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of publication.

CRediT authorship contribution statement

Ronny Vargas: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Catalina Lizano-Barrantes:** Visualization, Formal analysis, Data curation. **Miquel Romero:** Visualization, Formal analysis, Data curation. **Kevin Valencia-Clua:** Investigation. **David A. Narváez-Narváez:** Investigation. **Josep Ma Suñé-Negre:** Resources, Project administration. **Pilar Pérez-Lozano:** Resources, Project administration. **Encarna García-Montoya:** Resources, Project administration. **Noelia Martinez-Martinez:** Investigation. **Cristina Hernández-Munain:** Resources, Project administration. **Carlos Suñé:** Writing – review & editing, Supervision, Project administration. **Marc Suñé-Pou:** Writing – review & editing, Visualization, Supervision, Project administration, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Fig. 4-B Reprinted from Journal of the Taiwan Institute of Chemical Engineers, 110, Yung-Chih et al, Multiple-component dual-phase solid lipid nanoparticles with conjugated transferrin for formulating antioxidants and nerve growth factor against neuronal apoptosis, page 147, copyright 2020 with permission from Elsevier.

Fig. 6 Reprinted from Journal of Controlled Release, 328, Guo et al, Incorporation of docosahexaenoic acid (DHA) enhances nanodelivery of antiretroviral across the blood-brain barrier for treatment of HIV reservoir in brain, page 706, copyright 2020 with permission from Elsevier.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2024.124686.

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