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REVIEW ARTICLE

# Mechanisms of Active Targeting and Cellular Internalization of Surface-Engineered Lipid Nanocarriers for Site-Specific Oncological Interventions

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**Abstract**: Chemotherapeutic efficacy is frequently compromised by non-specific systemic distribution, multidrug resistance, and suboptimal intracellular accumulation within malignant tissues. Lipid-based nanocarriers, particularly liposomes and solid lipid nanoparticles, represent a dominant class of drug delivery systems due to their biocompatibility and capacity to encapsulate diverse hydrophobic and hydrophilic payloads. While first-generation nanocarriers relied on the Enhanced Permeability and Retention (EPR) effect for passive accumulation, clinical translation has highlighted significant heterogeneity in tumor vasculature permeability, necessitating a shift toward active targeting strategies. Functionalization of the nanocarrier surface with specific ligands including monoclonal antibodies, peptides, aptamers, and small molecules like folate facilitates direct interaction with overexpressed receptors on the tumor cell surface. This molecular recognition triggers receptor-mediated endocytosis, bypassing conventional diffusion barriers and enhancing the cytosolic bioavailability of the therapeutic agent. Critical physicochemical parameters, such as ligand density, tether length, and nanocarrier elasticity, fundamentally dictate the binding avidity and subsequent intracellular trafficking pathways. This review discusses about the transition of lipid nanocarriers from passive retention to active molecular targeting and the influence of surface architecture on cellular uptake kinetics. The optimization of these surface-engineered systems offers a pathway to maximize the therapeutic index while minimizing off-target cytotoxicity in precision oncology.

**Keywords:** Lipid nanocarriers; Active targeting; Surface functionalization; Receptor-mediated endocytosis; Tumor microenvironment.

## 1. Introduction

The fundamental challenge in conventional chemotherapy remains the inability to discriminate between neoplastic and healthy tissues, leading to dose-limiting systemic toxicity and a narrow therapeutic window. The advent of nanotechnology in pharmaceutics has provided a platform to alter the pharmacokinetic profile of cytotoxic agents significantly [1]. Among the diverse array of nanostructures developed, lipid-based nanocarriers comprising liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) have achieved the most substantial clinical success [2]. Their structural versatility allows for the protection of labile payloads from enzymatic degradation while reducing the requisite dosage through improved bioavailability.

Early advancements focused primarily on the prolongation of circulation half-life. The rapid clearance of circulating nanoparticles by the mononuclear phagocyte system (MPS), particularly the Kupffer cells of the liver and splenic macrophages, posed a significant barrier to effective tumor delivery [3]. The introduction of hydrophilic polymers, most notably polyethylene glycol (PEG), to the nanoparticle surface created a hydration layer that sterically hindered the adsorption of opsonins, thereby reducing phagocytic clearance. These "stealth" liposomes demonstrated superior circulation times, allowing them to exploit the pathophysiological characteristics of tumor vasculature [4]. However, long-circulation properties alone do not guarantee cellular internalization or drug release at the target site.

Current pharmaceutical research has pivoted toward the "third generation" of nanocarriers: active targeting. By decorating the surface of lipid carriers with targeting moieties that possess high affinity for receptors overexpressed on cancer cells, researchers aim to achieve site-specific delivery and enhanced cellular uptake [5]. This transition from passive accumulation to active recognition involves complex considerations regarding ligand-receptor dynamics, surface chemistry, and intracellular trafficking. This review

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delineates the mechanisms of active targeting, focusing on the chemical engineering of lipid nanocarriers and the biological imperatives that govern their efficacy in solid tumor therapy.

## 2. Hemodynamic Marginalization and the Enhanced Permeability and Retention (EPR)

To understand the necessity of active targeting, one must first analyze the limitations of passive targeting mechanisms. The passive accumulation of nanocarriers in solid tumors is largely attributed to the Enhanced Permeability and Retention (EPR) effect. Rapidly proliferating tumors induce angiogenesis, resulting in neovasculature that is structurally defective, characterized by wide fenestrations ranging from 200 nm to 2 μm, and a lack of smooth muscle distinct from the hierarchical architecture of normal blood vessels [6]. Concurrently, the lymphatic drainage in tumor tissues is often impaired or absent, preventing the efficient clearance of extravasated macromolecules.

**Table 1. Comparison of Passive and Active Targeting Techniques**

| Feature | Passive Targeting | Active Targeting |
|---|---|---|
| Primary Mechanism | Extravasation via leaky tumor vasculature (EPR effect). | Specific ligand-receptor interaction leading to internalization. |
| Driving Force | Hemodynamic pressure, diffusion, and impaired lymphatic drainage. | Molecular recognition and binding avidity (Kd). |
| Tumor Accumulation | Dependent on vascular fenestration size (200 nm – 2 μm) and cutoff. | Dependent on receptor expression density and accessibility. |
| Cellular Uptake | Generally low; nonspecific fluid-phase pinocytosis. | High; receptor-mediated endocytosis (Clathrin/Caveolae). |
| Primary Limitation | Heterogeneity of EPR effect; high interstitial fluid pressure. | Receptor saturation; "binding site barrier"; protein corona interference. |
| Main Determinants | Circulation half-life; PEGylation; Particle size (<200 nm). | Ligand density; Spacer length; Receptor recycling rate. |

Lipid nanocarriers within the size range of 50–200 nm can extravasate through these fenestrations and accumulate within the tumor interstitium [7]. While the EPR effect has been the cornerstone of nanomedicine for decades, recent clinical data suggests it is highly variable among patients and tumor types. Factors such as high interstitial fluid pressure (IFP) within the tumor core can oppose extravasation, limiting the penetration of nanoparticles to the tumor periphery [8]. Moreover, the dense extracellular matrix (ECM) composed of collagen and hyaluronic acid creates a steric barrier, restricting the diffusion of nanocarriers deep into the necrotic regions of the tumor. Consequently, reliance solely on passive targeting frequently results in suboptimal intracellular drug concentrations, necessitating an active targeting approach to facilitate cellular internalization once the carrier reaches the perivascular space [9].

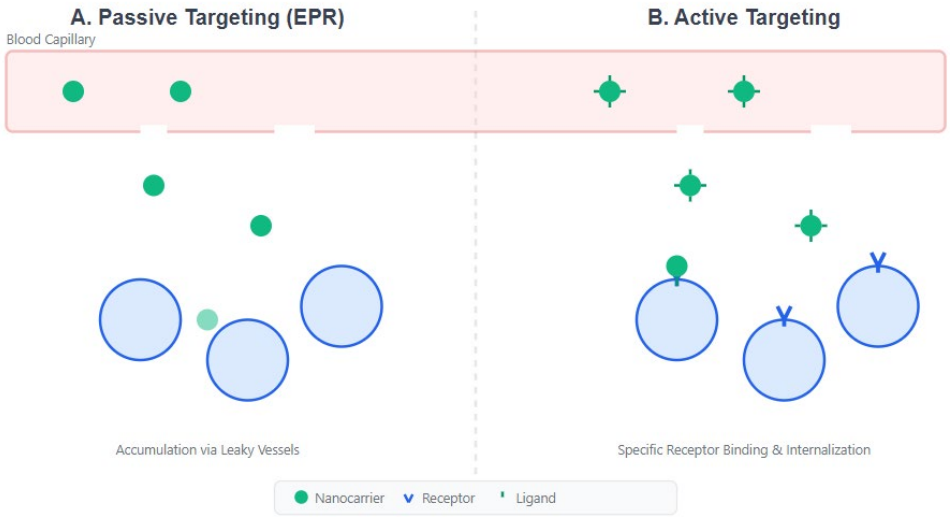


**Figure 1. Passive vs. Active Targeting Mechanisms**

## 3. Surface Engineering Strategies for Active Targeting

Active targeting relies on the specific interaction between a ligand attached to the nanocarrier surface and a receptor on the target cell. The engineering of these surfaces requires precise chemical strategies to ensure ligand stability, accessibility, and retention of biological activity.

### 3.1. Ligand Conjugation

The attachment of targeting moieties to the lipid surface is typically achieved through either post-insertion techniques or pre-functionalization of lipid components. The choice of conjugation chemistry is dictated by the functional groups available on the ligand (e.g., amines, thiols, carboxylic acids) and the desired orientation on the nanoparticle surface [10].

A prevalent method involves the formation of a thioether bond via the reaction between maleimide-functionalized lipids (e.g., DSPE-PEG-Maleimide) and thiol groups present on proteins or thiolated peptides. This reaction is highly specific at physiological pH and stable in serum conditions [11]. For ligands lacking free thiols, such as certain antibodies, thiolation can be introduced using Traut's reagent, though care must be taken to preserve the antigen-binding capability of the antibody. Alternatively, carbodiimide chemistry utilizing EDC/NHS (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide) facilitates the formation of amide bonds between carboxylated lipids and amine-containing ligands [12]. While effective, this method requires rigorous purification to remove unreacted crosslinkers and can lead to random conjugation orientations, potentially masking the ligand's active site.

**Table 2. Chemical Conjugation for Ligand Attachment**

| Chemistry | Reactive Groups Involved | Bond Formed | Advantages | Limitations |
|---|---|---|---|---|
| Maleimide-Thiol | Maleimide (Lipid) + Thiol (Ligand) | Thioether (Stable) | Highly specific at physiological pH (6.5–7.5); rapid reaction kinetics. | Requires free sulfhydryl groups on ligand; potential for oxidation of thiols. |
| Carbodiimide (EDC/NHS) | Carboxyl (Lipid) + Amine (Ligand) | Amide | Versatile; applicable to proteins and peptides without unique thiols. | Random conjugation orientation; risk of cross-linking/polymerization. |
| Click Chemistry (Cu-free) | Azide + DBCO (Cyclooctyne) | Triazole | Bioorthogonal; extremely high specificity; no catalyst required. | Synthetic complexity of DBCO-lipids; potential immunogenicity of cyclooctynes. |
| Post-Insertion | Amphiphilic Ligand-PEG conjugates | Hydrophobic interaction | Simple preparation; preserves ligand conformation. | Ligand retention stability depends on lipid anchor chain length. |

### 3.2. Impact of Spacer Length and Ligand Density

The spatial arrangement of ligands on the nanocarrier surface is a critical determinant of binding avidity. Ligands directly adsorbed or conjugated to the lipid bilayer surface may be shielded by the PEG corona used for steric stabilization, rendering them inaccessible to the target receptor [13]. To mitigate this "steric hindrance" effect, ligands are frequently tethered to the distal end of PEG chains. The molecular weight of the PEG spacer influences the ligand's conformational freedom and its ability to penetrate the receptor binding pocket. Studies indicate that a PEG spacer length corresponding to a molecular weight of 2000–3400 Da often provides an optimal balance between steric stabilization and ligand accessibility [14].

Moreover, ligand density plays a pivotal role in the "multivalency" effect. A higher density of ligands can lead to multivalent binding, where multiple ligand-receptor interactions occur simultaneously, exponentially increasing the binding avidity compared to a single interaction [15]. However, an excessively high ligand density can be counterproductive. It may induce the "hook effect" or immune recognition, accelerating clearance by the reticuloendothelial system (RES) before the carrier reaches the tumor site. Therefore, an optimized ligand density, typically ranging from 2% to 5% molar ratio of the total lipid content, is often required to maximize tumor uptake while maintaining long-circulation properties [16].

## 4. Ligand-Receptor Targeting Modalities

The selection of an appropriate targeting ligand is paramount to the success of active targeting strategies. The ideal ligand must exhibit high affinity for a receptor that is exclusively or preferentially expressed on tumor cells relative to normal tissue. Moreover, the receptor density on the cell surface must be sufficient to trigger efficient endocytosis upon ligand binding. Various classes of ligands, ranging from small molecules to complex proteins, have been investigated for surface engineering of lipid nanocarriers.

### 4.1. Small Molecule Targeting: The Folate Receptor

Small molecules represent a distinct advantage in active targeting due to their low molecular weight, high stability, low immunogenicity, and relative ease of conjugation. Among these, folic acid (vitamin B9) remains one of the most extensively studied ligands. The folate receptor alpha (FRα) is a glycosylphosphatidylinositol-anchored protein that is significantly upregulated in numerous epithelial malignancies, including ovarian, breast, lung, and renal cancers, while being restricted to the apical surface of polarized epithelia in normal tissues [17].

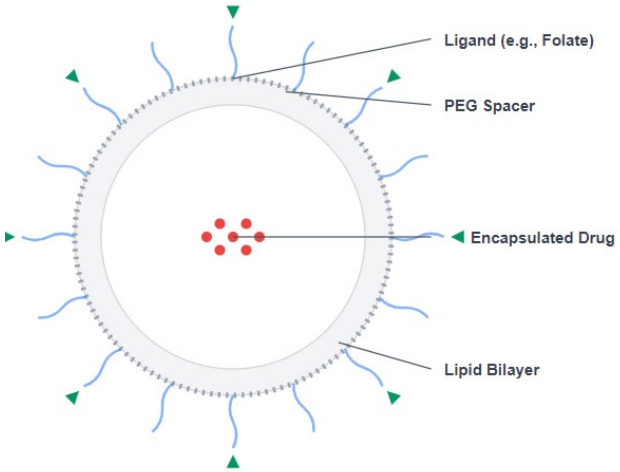


**Figure 2. Structure of a Ligand-Targeted Liposome**

Folic acid binds to FRα with a high affinity (Kd ~ $10^{-10}$ M), a binding strength comparable to antigen-antibody interactions. Upon binding, the folate-conjugated nanocarrier is internalized via receptor-mediated endocytosis. A significant physiological advantage of folate targeting is the receptor's ability to recycle back to the cell surface after releasing its cargo in the endosome, allowing for a continuous cycle of drug uptake [18]. However, the ubiquity of endogenous folate in the bloodstream can potentially compete with folate-functionalized nanocarriers for receptor binding. Despite this, studies have demonstrated that the multivalent presentation of folate on the liposomal surface often overcomes this competition through higher avidity interactions [19]. Chemical conjugation is typically achieved by linking folic acid to the distal end of a PEG-lipid anchor (e.g., Folate-PEG-DSPE), ensuring the ligand extends beyond the steric barrier to interact with the receptor.

**Table 3. Ligand Classes Employed in Active Tumor Targeting**

| Ligand Class | Examples | Target Receptor | Characteristics |
|---|---|---|---|
| Small Molecules | Folic Acid (Folate) | Folate Receptor α (FRα) | High affinity (Kd ~$10^{-10}$ M); non-immunogenic; stable; low cost. |
| Proteins | Transferrin | Transferrin Receptor (TfR1) | Efficient internalization; crosses Blood-Brain Barrier (BBB). |
| Antibodies (mAbs) | Trastuzumab, Cetuximab | HER2, EGFR | High specificity and avidity; well-characterized clinical profiles. |
| Antibody Fragments | Fab', scFv, Nanobodies | Various Antigens | Reduced immunogenicity; lacks Fc region; smaller hydrodynamic radius. |
| Peptides | RGD (Arg-Gly-Asp) | Integrins (αv, β3) | Targets tumor neovasculature; easy to synthesize and functionalize. |

### 4.2. Protein-Based Targeting: Transferrin and Antibodies

Macromolecular ligands offer high specificity but present challenges regarding stability and immunogenicity. Transferrin (Tf), an 80 kDa iron-transport glycoprotein, targets the transferrin receptor (TfR1), which is ubiquitously expressed but upregulated up to 100-fold in proliferating malignant cells due to their increased iron demand [20]. Transferrin-conjugated lipid nanoparticles have demonstrated enhanced uptake in brain gliomas, as TfR is also highly expressed on the blood-brain barrier (BBB) endothelium. A limiting factor for Tf-targeting is the high concentration of endogenous transferrin in plasma (approx. 25 μM), which saturates Tf receptors. Consequently, recent strategies have shifted towards using anti-TfR antibodies or peptides that bind to allosteric sites on the receptor distinct from the native transferrin binding pocket, thereby avoiding competitive inhibition [21].

Monoclonal antibodies (mAbs) provide unparalleled specificity for tumor-associated antigens such as HER2 (human epidermal growth factor receptor 2) and EGFR (epidermal growth factor receptor). Trastuzumab-modified liposomes, for instance, have shown superior efficacy in HER2-positive breast cancer models compared to non-targeted counterparts [22]. Despite their specificity, whole antibodies significantly increase the hydrodynamic diameter of the nanocarrier and can trigger complement activation or uptake by the Fc-receptor-bearing immune cells. To mitigate this, antibody fragments such as Fab' or single-chain variable fragments (scFv) are increasingly utilized. These fragments retain the antigen-binding domain while eliminating the Fc region, thus reducing immunogenicity and MPS clearance while maintaining targeting efficacy [23].

## 5. Cellular Internalization and Intracellular Trafficking

The successful binding of a targeted nanocarrier to the tumor cell surface is merely the precursor to the critical step of intracellular delivery. The therapeutic payload must not only enter the cell but also reach the appropriate subcellular compartment to exert its cytotoxic effect.

### 5.1. Receptor-Mediated Endocytosis Pathways

Following ligand-receptor binding, lipid nanocarriers are typically internalized via receptor-mediated endocytosis. The specific pathway utilized clathrin-mediated, caveolae-mediated, or macropinocytosis dictates the subsequent intracellular fate of the carrier. Clathrin-mediated endocytosis is the most common route for ligands such as transferrin and folate. In this process, the membrane invaginates to form a clathrin-coated pit, which scissions to form an early endosome [24]. This compartment is characterized by a gradual drop in pH from physiological levels (7.4) to approximately 6.0–6.5.

Conversely, carriers targeting caveolin-enriched microdomains (lipid rafts) may enter via caveolae-mediated endocytosis. This pathway is particularly advantageous for bypassing lysosomal degradation, as caveosomes are distinct non-acidic compartments that can transport cargo directly to the Golgi apparatus or the endoplasmic reticulum [25]. The physicochemical properties of the nanocarrier, particularly size and surface charge, influence the pathway selection. Larger particles (>200 nm) may preferentially trigger macropinocytosis or caveolae-mediated uptake, while smaller particles (<150 nm) are favored by clathrin-dependent mechanisms.

### 5.2. The Endosomal Barrier and Cytosolic Delivery

A major bottleneck in the efficacy of targeted lipid nanocarriers is entrapment within endosomes and subsequent degradation in lysosomes. As the early endosome matures into a late endosome and eventually fuses with a lysosome, the pH drops to roughly 4.5, and hydrolytic enzymes are activated. For therapeutics requiring cytosolic access (e.g., siRNA, mRNA, or certain chemotherapeutics), escape from the endosome prior to lysosomal fusion is imperative [26]. To overcome this barrier, "smart" lipid nanocarriers are engineered with pH-sensitive components. One common strategy incorporates fusogenic lipids, such as dioleoylphosphatidylethanolamine (DOPE). Under physiological conditions, DOPE forms stable bilayers when stabilized by other lipids. However, in the acidic environment of the endosome, DOPE undergoes a phase transition from a lamellar to an inverted hexagonal structure (HII phase). This transition disrupts the endosomal membrane, facilitating the release of the encapsulated payload into the cytoplasm [27]. Another approach involves the use of pH-sensitive titratable polymers or lipids that become protonated at endosomal pH. This protonation leads to the "proton sponge" effect, causing an influx of chloride ions and water, resulting in osmotic swelling and rupture of the endosome. Surface-engineered nanocarriers can ensure that the enhanced uptake achieved through active targeting translates into genuine therapeutic efficacy by combining these escape mechanisms.

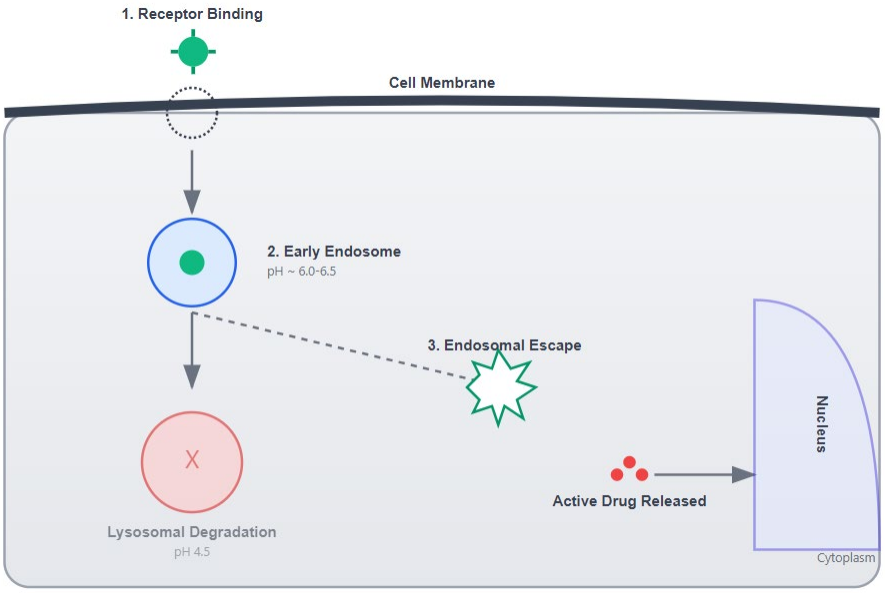


**Figure 3. Intracellular Trafficking & Endosomal Escape**

**Table 4. Mechanisms of Endosomal Escape for Cytosolic Delivery**

| Mechanism | Component/Lipid | Trigger | Process Description |
|---|---|---|---|
| Membrane Fusion/Destabilization | DOPE (Dioleoylphosphatidylethanolamine) | Acidic pH (5.0–6.0) | Protonation causes transition from lamellar (Lα) to inverted hexagonal (HII) phase, disrupting the endosomal membrane. |
| Proton Sponge Effect | PEI (Polyethylenimine), Histidine-rich lipids | Acidic pH | Buffering capacity leads to massive proton and $Cl^-$ influx, causing osmotic swelling and endosomal rupture. |
| Pore Formation | Cell-Penetrating Peptides (e.g., TAT, GALA) | pH or Membrane Potential | Peptides insert into the lipid bilayer to form transient pores, allowing payload leakage. |
| Photosensitization | Photosensitizers (e.g., TPPS 2a) | Light (PCI - Photochemical Internalization) | Light activation generates ROS that specifically oxidize endosomal membrane lipids, causing leakage. |

## 6. Challenges to Clinical Translation and the "Active Targeting Paradox"

Despite the robust accumulation of preclinical data demonstrating the superiority of ligand-functionalized nanocarriers over their non-targeted counterparts, the translation of these technologies into approved clinical therapies has been paradoxically slow. This discrepancy, often termed the "active targeting paradox," arises from complex biological interactions that occur once the nanocarrier enters the systemic circulation. A primary impediment is the formation of the protein corona. Upon intravenous administration, lipid nanoparticles are immediately coated by a complex layer of serum proteins, including albumin, apolipoproteins, and complement factors [28]. This adsorbed protein layer can sterically mask the targeting ligands, rendering them inaccessible to the tumor cell receptors. Consequently, the distinct recognition capabilities engineered in vitro may be significantly dampened in the in vivo environment.

Moreover, the heterogeneity of human tumors presents a substantial barrier. Unlike immortalized cell lines used in preclinical models, which often express uniform high levels of specific receptors, clinical tumors exhibit significant inter- and intra-tumoral heterogeneity. Receptor expression levels can vary widely between the primary tumor and metastatic lesions, or even among cells within the same tumor mass [29]. If the target receptor is downregulated or absent in a subpopulation of cancer cells, those cells may escape treatment, leading to disease recurrence. Additionally, the stringent manufacturing controls required for complex, multi-component nanocarriers termed Chemistry, Manufacturing, and Controls (CMC) pose significant regulatory hurdles. Ensuring batch-to-batch consistency regarding ligand orientation, density, and drug encapsulation efficiency is exponentially more difficult for active targeting systems than for passive "stealth" liposomes.

**Table 5. Selected Active Targeted Lipid Nanocarriers in Clinical Development**

| Candidate Name | Targeting Ligand | Payload | Target Indication | Status/Observation |
|---|---|---|---|---|
| SGT-53 | Anti-TfR scFv | p53 DNA plasmid | Solid Tumors (Glioblastoma) | Phase II. Demonstrated accumulation in metastatic lesions. |
| MBP-426 | Transferrin | Oxaliplatin | Gastroesophageal Cancer | Phase II. Improved tolerability compared to free oxaliplatin. |
| 2B3-101 | Glutathione (G-Technology) | Doxorubicin | Brain Metastases | Phase I/II. Glutathione targets transporters at the BBB. |
| MM-302 | Anti-HER2 scFv | Doxorubicin | HER2+ Breast Cancer | Phase II (Discontinued). Showed activity but failed to surpass standard of care (T-DM1). |
| C225-ILs-Dox | Cetuximab (Anti-EGFR) | Doxorubicin | Advanced Solid Tumors | Phase I. Proof of concept for EGFR targeting with immunoliposomes. |

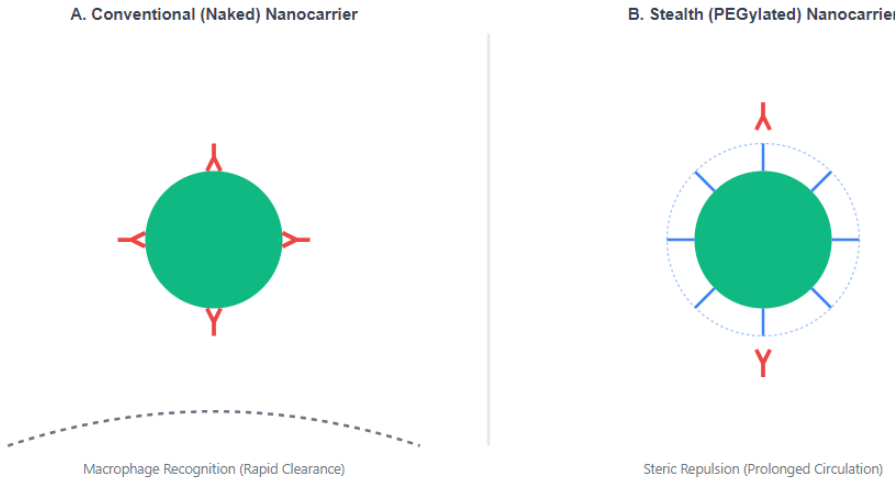


**Figure 4. The Stealth Effect (PEGylation)**

## 7. Dual-Targeting and Stimuli-Responsive Systems

To address the limitations of single-ligand targeting and heterogeneous receptor expression, the next generation of lipid nanocarriers is moving toward dual-targeting strategies. By conjugating two distinct ligands that target different receptors (e.g., transferrin and folate, or an integrin antagonist and an antibody), researchers aim to increase the probability of cellular recognition and overcome receptor saturation or downregulation [30]. This approach also holds promise for crossing physiological barriers; for instance, one ligand may facilitate transport across the blood-brain barrier while the second targets the glioblastoma cells specifically.

Concurrently, the integration of active targeting with stimuli-responsive mechanisms represents a frontier in precision oncology. These "smart" systems are designed to remain stable during circulation but undergo a physicochemical change such as disassembly or membrane destabilization in response to intrinsic tumor stimuli (acidic pH, elevated redox potential, or specific enzymes) or extrinsic triggers (ultrasound, magnetic fields, or light). The convergence of active molecular recognition with environmentally triggered release profiles aims to achieve spatiotemporal control over drug delivery, maximizing the therapeutic index.

## 8. Conclusion

The evolution of lipid nanocarriers from passive vessels relying on hemodynamic retention to sophisticated, active targeting systems marks a pivotal advancement in oncological pharmacotherapy. While the Enhanced Permeability and Retention effect provides the foundational access to the tumor microenvironment, it is the surface engineering of these carriers with specific ligands that dictates cellular fate and intracellular bioavailability. The strategic selection of ligands, optimization of spacer length, and control over surface density are critical parameters that influence binding avidity and internalization pathways. However, the biological reality of the protein corona and tumor heterogeneity necessitates a nuanced approach to nanocarrier design.

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