



Review Article

JOURNAL OF APPLIED PHARMACOLOGY AND TOXICOLOGY | JOAPT
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EXPLOITING RECEPTOR NANOCLUSTERS TO BIAS CLATHRIN VS. CAVEOLIN UPTAKE: MOLECULAR DESIGN RULES FOR TARGETED ANTI-CANCER NANOMEDICINES

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Article Information

Received: 15th March 2026

Revised: 2th May 2026

Accepted: 5th June 2026

Published: 30th June 2026

Keywords

Endocytic, caveolin, clathrin, endocytosis, computational modeling

ABSTRACT

Targeted nanomedicine has advanced beyond cell-specific binding toward the deliberate programming of cellular entry pathways. A central determinant of endocytic pathway selection is receptor nano-clustering, a dynamic process governed by multivalent ligand interactions, membrane lipid organization, and cytoskeletal constraints. This work outlines molecular design principles for engineering nanoparticles that bias uptake toward clathrin-mediated or caveolin-mediated endocytosis. Evidence across model systems suggests that nanoparticles in the 30-80 nm size range with moderate-to-high ligand densities (~ 10 -100 ligands per particle or ~ 0.01 -0.1 ligands nm^{-2}) typically promote receptor aggregation sufficient for clathrin-mediated endocytosis (CME) through adaptor protein recruitment and progressive membrane curvature. Particles of approximately 50-150 nm, particularly those presenting lower-to-intermediate ligand densities (~ 0.001 -0.05 ligands nm^{-2}) and broader spatial ligand separation, more frequently engage caveolin-mediated endocytosis (CvME) within cholesterol-rich membrane domains, facilitating intracellular delivery while reducing lysosomal exposure. Beyond size and ligand density, nanoparticle shape, ligand spacing (~ 10 -30 nm inter-ligand distance), and receptor-ligand residence time collectively regulate clustering geometry and pathway stability. Clathrin-mediated uptake supports efficient internalization but often directs cargo toward degradative endo-lysosomal compartments; caveolin-associated entry may preserve the integrity of sensitive therapeutic payloads. Integration of quantitative imaging, computational modeling, and microenvironmental factors enables predictive control over intracellular trafficking. These insights establish receptor nano-clustering as a tunable design variable for optimizing therapeutic delivery and achieving pathway-selective enhancement of anti-cancer nanomedicines.

INTRODUCTION

The plasma membrane is not merely a passive cellular boundary but a dynamic biochemical interface that governs the recognition, internalization, and intracellular trafficking of

exogenous materials. In targeted nanomedicine, it functions simultaneously as a biological barrier and a programmable gateway that directs nanoparticles into mechanistically distinct

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uptake pathways [1]. Among these, clathrin-mediated endocytosis (CME) and caveolin-mediated endocytosis (CvME) are particularly important because they differ in vesicle architecture, membrane mechanics, cargo sorting, and intracellular fate. CME generally directs cargo toward endolysosomal degradation, whereas CvME frequently mediates non-degradative trafficking that preserves sensitive therapeutic payloads. Despite significant advances in targeted nanotherapeutics, current literature remains fragmented, emphasizing receptor targeting, ligand chemistry, or intracellular trafficking independently while providing limited mechanistic insight into how receptor nanoscale organization governs pathway selection [3]. Conventional nanoparticle design largely treats receptor binding as a binary event without considering the spatial, temporal, and cooperative organization of receptors within the plasma membrane [4]. Although membrane biophysics, lipid raft dynamics, and multivalent interactions have each been extensively studied, researchers rarely integrate them into a predictive framework for engineering pathway-selective uptake [5]. A major mechanistic gap therefore lies in understanding how receptor nano-clustering couples membrane biophysics with endocytic machinery recruitment to determine intracellular trafficking fate. Cellular receptors exist as dynamic nanoscale assemblies regulated by lipid phase organization, membrane elasticity, cytoskeletal confinement, and cooperative receptor-ligand interactions [6]. Nanoparticle-induced receptor crosslinking reorganizes these assemblies into distinct energetic states that selectively recruit clathrin adaptors or caveolar scaffolds [7]. However, the principles linking nanoparticle physicochemical properties to receptor nano-organization remain insufficiently systematized, resulting in largely empirical nanoparticle optimization.[8] This review addresses this gap by integrating receptor nano-organization, membrane biophysics, and nanoparticle engineering into a unified mechanistic framework for pathway-selective nanomedicine design. We present receptor nanoclustering as a programmable engineering variable that can control intracellular trafficking outcomes [9]. By combining multivalent binding, membrane energetics, lipid-domain organization, and endocytic machinery recruitment, we establish predictive molecular design principles linking nanoparticle size, shape, ligand density, ligand spacing, and surface mechanics to selective engagement of CME or CvME, thereby enabling control over intracellular destination, cargo preservation, and therapeutic efficacy [10].

1.1 From passive targeting to pathway-specific design

Early anti-cancer nanotherapeutics primarily exploited the enhanced permeability and retention (EPR) effect to improve tumor accumulation. Although successful at the tissue level, this strategy did not address cellular internalization mechanisms. Ligand-functionalized nanoparticles subsequently improved receptor-mediated uptake specificity but still treated receptor engagement largely as a static binding event [13]. Receptor engagement now dynamically reorganizes the plasma membrane at nanometre scales [14]. Multivalent nanoparticles induce receptor crosslinking, stabilize nanoscale assemblies and promote cooperative clustering that serves as a platform for recruiting distinct endocytic machineries [15]. Consequently, nanomedicine has shifted from selective cell targeting to programming intracellular entry routes and trafficking outcomes [16].

1.2 Molecular consequences of endocytic pathway selection

The distinction between CME and CvME has major therapeutic implications. CME begins with adaptor-mediated recognition of sorting motifs followed by clathrin lattice assembly that drives membrane curvature and vesicle formation [17]. This pathway is tightly coupled to endo-lysosomal maturation, where acidification and enzymatic degradation can compromise proteins, peptides, and nucleic acids [18]. Conversely, CvME operates within cholesterol- and sphingolipid-rich lipid rafts [19]. Caveolae, stabilized by caveolin-1 and cavin complexes, internalize cargo into vesicles that frequently bypass lysosomal degradation [20]. Caveolar trafficking can direct cargo toward caveosomes, the endoplasmic reticulum or transcytotic pathways, thereby preserving therapeutic integrity [21]. These distinct intracellular fates arise from differences in membrane composition, curvature-generation mechanisms and scaffold architecture, all of which are strongly influenced by receptor spatial organization [22].

1.3 Receptor nano-clustering as a central design principle

Receptor nano-clustering provides the mechanistic link between nanoparticle engineering and endocytic pathway selection. Cell-surface receptors form dynamic membrane assemblies whose size, density, and lifetime are regulated by lipid organization, protein interactions, and cytoskeletal confinement [23]. Multivalent nanoparticle binding promotes cooperative receptor crosslinking and cluster stabilization [24]. Cluster formation reflects a balance between favorable ligand-receptor binding

energy and energetic penalties associated with membrane deformation, receptor confinement, and entropy loss [25]. By lowering the free-energy barrier for membrane curvature generation, multivalent nanoparticles template vesicle formation [26]. The resulting cluster geometry determines pathway preference: smaller, transient clusters favor adaptor-mediated clathrin assembly, whereas larger, raft-associated and mechanically stable clusters preferentially recruit caveolar machinery [27].

1.4 Toward predictive control of cellular entry

Engineering nanoparticle size, shape, ligand density, ligand spacing and surface mechanics enables modulation of receptor clustering and selective biasing of intracellular uptake pathways [28]. This represents a transition from empirical optimization toward predictive engineering of cellular entry based on membrane biophysics [29]. Within this framework, the plasma membrane is viewed as an active, responsive system whose

behavior can be quantitatively programmed through molecular design [30]. Such control is particularly important in oncology, where therapeutic efficacy depends on intracellular localization. Cytosolic delivery of siRNA, preservation of protein therapeutics and nuclear delivery of gene-editing systems all benefit from selective engagement of non-degradative pathways [31]. Accordingly, this review systematically examines the molecular and biophysical principles governing receptor nano-organization, membrane energetics and endocytic pathway selection and develops quantitative nanoparticle design rules for selectively directing CME or CvME in targeted anti-cancer nanomedicine [32]. Figure 1 illustrates how receptor nano-clustering and multivalent ligand interactions regulate endocytic pathway selection. Small, transient clusters favor clathrin-mediated endocytosis, whereas large, stable lipid raft-associated clusters promote caveolin-mediated uptake. Membrane curvature and cluster size collectively determine intracellular trafficking fate and vesicle formation.

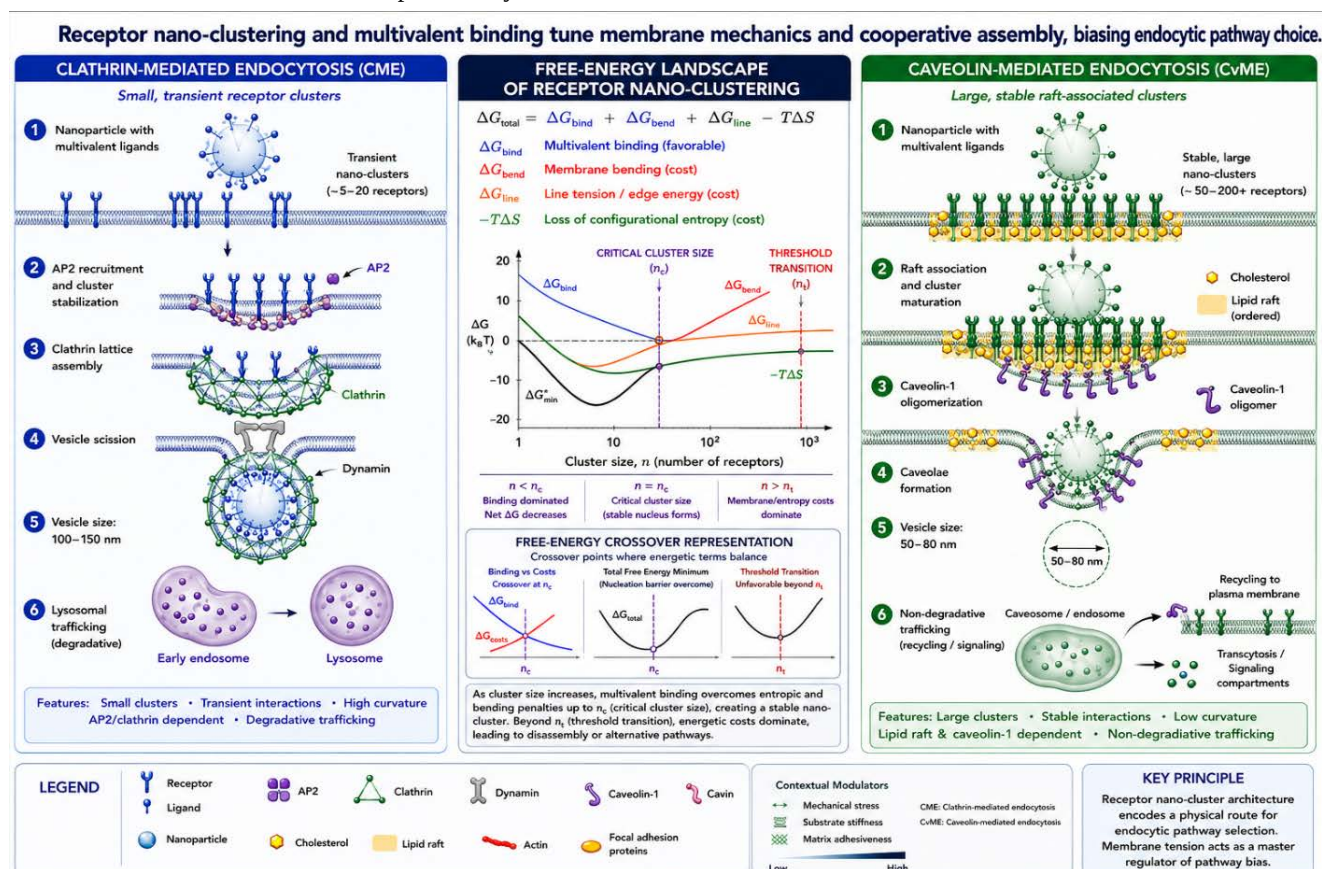


Figure 1: Receptor nano-cluster architecture governs endocytic pathway selection

(Mechanistic illustration of receptor nano-clustering-mediated endocytic pathway selection. Small, transient receptor clusters favor clathrin-mediated endocytosis (CME), whereas large, stable lipid raft-associated clusters promote caveolin-mediated endocytosis (CvME). The central free-energy landscape highlights the critical cluster size (n_c), threshold transition (n_t), and free-energy crossover that govern pathway bias through the balance between multivalent binding, membrane bending, line tension, and entropy.)

1.5 Literature search and article selection methodology

This review was developed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure methodological transparency, reproducibility, and minimization of selection bias. We conducted a systematic literature search using PubMed, Scopus, Web of Science, and Google Scholar to identify relevant studies on receptor nano-clustering, membrane biophysics, endocytic pathway selection, and nanoparticle engineering strategies for targeted anti-cancer nanomedicine. The literature search was restricted to studies published between January 2000 and March 2025 to capture contemporary advances in nanoscale membrane organization, pathway-selective intracellular delivery, and predictive nanoparticle engineering. Search terms and Boolean combinations included: receptor nano-clustering, clathrin-mediated endocytosis, caveolin-mediated endocytosis, lipid raft-mediated uptake, nanoparticle internalization, multivalent ligand interactions, membrane curvature, endocytic pathway bias, targeted nanomedicine, intracellular trafficking, and tumor-targeted nanoparticles. We identified additional studies by manually screening reference lists from relevant reviews and primary research articles.

Studies were included if they met the following criteria:

- (i) investigated molecular, cellular or biophysical mechanisms underlying receptor clustering or endocytic pathway regulation;
- (ii) examined nanoparticle physicochemical parameters including size, shape, ligand density, ligand spacing, surface chemistry or membrane interactions;
- (iii) reported experimental, computational or imaging-based evidence relevant to clathrin-mediated or caveolin-mediated uptake; and
- (iv) focused on mammalian cellular systems, cancer nanomedicine or intracellular therapeutic delivery applications.

Studies were excluded if they:

- (i) lacked direct mechanistic relevance to receptor nano-organization or pathway-selective endocytosis;
- (ii) focused exclusively on non-mammalian or environmentally unrelated systems;
- (iii) were conference abstracts, editorials, opinions or non-peer-reviewed reports lacking sufficient methodological detail; or
- (iv) contained incomplete, non-reproducible or insufficiently validated data.

After database retrieval, we removed duplicate records and screened titles and abstracts for relevance. We then evaluated

eligible articles in full text. We prioritized studies that provided quantitative or mechanistic evidence linking nanoparticle architecture to receptor clustering dynamics, membrane mechanics, and intracellular trafficking behavior. We placed particular emphasis on investigations integrating membrane biophysics, multivalent interactions, and rational nanoparticle engineering principles. The final article selection process followed PRISMA-guided stages of identification, screening, eligibility, and inclusion to ensure a systematic and scientifically balanced synthesis of recent advances in pathway-selective nanomedicine design.

2. PLASMA MEMBRANE NANO-ORGANIZATION

The plasma membrane is a structurally complex and functionally heterogeneous assembly whose nanoscale organization governs receptor behavior and endocytic pathway selection [19]. Rather than a uniform bilayer, it is a dynamically compartmentalized system in which lipids and proteins segregate into transient yet functionally significant nano-domains. These domains provide the biophysical environment in which receptor nanoclusters form and recruit distinct endocytic machineries [20].

2.1 Lipid heterogeneity and lateral phase behavior

Membrane heterogeneity arises from differential lipid packing. Saturated phospholipids, sphingolipids, and cholesterol assemble into liquid-ordered (L_o) phases characterized by tight packing, reduced lateral diffusion, and increased membrane thickness, whereas unsaturated phospholipids form more fluid liquid-disordered (L_d) phases [21]. Their coexistence creates a lateral membrane landscape that selectively partitions proteins according to physicochemical compatibility. Cholesterol is a central regulator of membrane order and curvature elasticity [22]. Within raft-like domains, it stabilizes sphingolipid-rich assemblies that concentrate proteins such as glycosylphosphatidylinositol (GPI)-anchored proteins and certain integrins. These dynamic domains, typically 10-200 nm in size and persisting from microseconds to seconds, rapidly reorganize in response to ligand binding [23].

2.2 Receptor nanoclusters: formation and dynamics

Cell-surface receptors rarely diffuse as isolated molecules; instead, they exist in pre-organized nanoclusters or rapidly cluster after ligand engagement [24]. In nanomedicine, multivalent nanoparticles drive this process by simultaneously engaging multiple receptors, producing cooperative crosslinking, increasing local receptor density, and promoting

receptor oligomerization [25]. Cluster stability depends primarily on binding avidity rather than individual ligand affinity. The actin cytoskeleton provides an additional level of spatial regulation by partitioning the membrane into diffusion-limited corrals that increase receptor encounters and cluster formation [26]. Dynamic actin remodeling can either stabilize or disperse receptor assemblies, coupling membrane organization to cellular signaling and mechanical state [27].

2.3 Classes of receptors relevant to cancer nanomedicine

Distinct receptor families display characteristic clustering behaviors that influence endocytic pathway selection. Receptor tyrosine kinases (RTKs), including EGFR and HER2, undergo ligand-induced dimerization and higher-order clustering that frequently promotes clathrin-mediated internalization [28]. Integrins are strongly regulated by lipid rafts and cytoskeletal interactions, enabling adhesion-dependent clustering that can favor caveolar uptake during multivalent engagement. GPI-anchored receptors preferentially partition into ordered lipid domains, making them closely associated with caveolin-mediated endocytosis [29]. Scavenger receptors and selected G protein-coupled receptors (GPCRs) further broaden the range of clustering dynamics that can be exploited for nanoparticle targeting [30].

2.4 Energetic landscape of nano-cluster formation

Receptor nano-clustering is governed by the balance of competing thermodynamic forces that determine the total free energy change (ΔG_{total}) [31]. Favorable ligand-receptor

binding energy (ΔG_{bind}), amplified through multivalent interactions, competes with membrane bending energy (ΔG_{bend}), line tension at lipid-domain boundaries (ΔG_{line}), and the entropic penalty ($-T\Delta S$) associated with receptor confinement [32]. Multivalent nanoparticles generate sufficient binding energy to overcome membrane deformation and entropy costs, creating stable receptor clusters. Nanoparticle size, curvature, and ligand spacing strongly influence this free-energy landscape by dictating receptor organization and cluster stability [33]. These cooperative thermodynamic contributions provide the quantitative basis for membrane bending, multivalent-binding avidity, and total free-energy models that predict receptor nano-clustering and subsequent endocytic pathway selection. The membrane bending energy described below is commonly represented using the Helfrich curvature formalism, where the energetic cost of membrane deformation depends on membrane bending rigidity (κ), local membrane curvature (H), and spontaneous curvature (C_0). The total free energy of cluster formation is determined by the balance between favorable ligand-receptor binding interactions and opposing energetic penalties arising from membrane deformation, line tension, and entropy loss. In addition, multivalent ligand presentation enhances effective receptor binding through avidity effects, where the cumulative interaction strength increases with the number of simultaneously engaged receptors. Together, these relationships provide a quantitative biophysical basis for understanding how nanoparticle architecture modulates receptor organization and stabilizes pathway-selective endocytic nanoclusters.

1. Membrane bending energy:

$$E_{bend} = \frac{\kappa}{2} \int (2H - C_0)^2 dA$$

E_{bend} : Membrane bending energy
 κ : Bending rigidity (in J)
 H : Mean curvature (1/m)
 C_0 : Spontaneous curvature (1/m)
 dA : Differential membrane area

2. Free energy relationship:

$$\Delta G_{total} = \Delta G_{bind} + \Delta G_{bend} + \Delta G_{line} - T\Delta S$$

ΔG_{total} : Total free energy change
 ΔG_{bind} : Binding free energy
 ΔG_{bend} : Membrane bending energy
 ΔG_{line} : Line tension energy
 T : Absolute temperature (K)
 ΔS : Entropy change

3. Multivalent avidity relationship:

$$K_{avidity} \propto nK_a$$

$K_{avidity}$: Avidity constant
 n : Number of simultaneously engaged ligands
 K_a : Monovalent affinity constant

2.5 Coupling between nano-organization and endocytic competence

Receptor nanoclusters are not merely passive assemblies; they actively recruit endocytic machinery. A cluster's size, density,

and lipid context determine its compatibility with specific adaptor proteins and scaffolding complexes [34]. Clusters enriched in phosphatidylinositol 4,5-bisphosphate (PIP₂) and containing receptors with canonical sorting motifs are primed for

clathrin adaptor binding. Clusters residing within cholesterol-rich, ordered domains are predisposed to interact with caveolin-1 and associated proteins. Small changes in cluster size or composition can disproportionately affect pathway selection [35]. The membrane's nanoscale organization acts as a critical intermediary between nanoparticle design and cellular response. By modulating the parameters that govern cluster formation, most notably ligand valency, spatial distribution, and nanoparticle curvature, it becomes possible to steer the membrane toward a desired endocytic outcome [36].

3. CLATHRIN-MEDIATED ENDOCYTOSIS (CME): MOLECULAR MACHINERY

Clathrin-mediated endocytosis (CME) is one of the best-characterized and evolutionarily conserved mechanisms of cellular internalization. It is a highly coordinated process in which cargo selection, membrane deformation, and vesicle formation are regulated through sequential protein-lipid and protein-protein interactions [37]. CME provides an efficient route for receptor-mediated uptake while predominantly directing cargo toward the endo-lysosomal system, creating both opportunities for cellular delivery and challenges associated with intracellular degradation [38].

3.1 Initiation: nucleation of endocytic sites

CME is initiated by localized enrichment of phosphatidylinositol 4,5-bisphosphate (PIP₂), which recruits the adaptor protein complex AP2 [39]. AP2 binding to PIP₂ and cargo receptors induces a conformational change that exposes binding sites for both cargo and clathrin. AP2 recognizes canonical tyrosine-based and dileucine sorting motifs within receptor cytoplasmic domains, ensuring selective incorporation into nascent clathrin-coated pits [40]. In nanomedicine, multivalent nanoparticle-induced receptor clustering increases the local density of these internalization motifs, thereby promoting endocytic site nucleation [41].

3.2 Coat assembly: clathrin lattice formation and membrane curvature

Clathrin triskelions assemble into a polyhedral lattice of hexagonal and pentagonal units that progressively generates membrane curvature and forms vesicles approximately 100-150 nm in diameter [42]. Membrane bending is facilitated by BAR-domain proteins, which stabilize curved membranes, and epsin, whose amphipathic helices insert into the lipid bilayer to reduce the energetic barrier for invagination [43]. Together, these

proteins drive the transition from a planar membrane to a deeply invaginated clathrin-coated pit [44].

3.3 Vesicle scission: dynamin-mediated membrane fission

Vesicle release is mediated by the GTPase dynamin, which assembles around the neck of the budding vesicle. GTP hydrolysis induces conformational changes that constrict the neck and produce membrane fission, directly coupling chemical energy to mechanical force [45]. Accessory proteins including amphiphysin and endophilin stabilize the highly curved neck and facilitate dynamin assembly, resulting in release of the clathrin-coated vesicle into the cytoplasm [46].

3.4 Uncoating and intracellular trafficking

After internalization, heat shock cognate protein 70 (Hsc70) and auxilin rapidly remove the clathrin coat, enabling vesicle fusion with early endosomes [47]. Progressive acidification within the endo-lysosomal pathway promotes ligand-receptor dissociation and activation of hydrolytic enzymes. Although advantageous for applications such as prodrug activation, this degradative environment limits the intracellular delivery of proteins, peptides and nucleic acids [48].

3.5 Molecular determinants of CME efficiency

CME efficiency depends on coordinated regulation of receptor density, ligand valency, membrane mechanics and cytoskeletal dynamics. Increased receptor clustering enhances AP2 recruitment and stabilizes clathrin-coated pit formation, whereas excessive clustering may alter pathway selection [49]. Membrane tension opposes curvature generation, while actin polymerization supplies mechanical support for invagination, particularly under high-tension conditions. Nanoparticle physicochemical properties strongly influence these factors [50].

3.6 Implications for nanomedicine design

CME is well suited for rapid internalization of small-molecule therapeutics and cargos that remain functional within endosomal compartments. Nanoparticles smaller than approximately 50 nm, displaying moderate ligand density and targeting receptors bearing canonical internalization motifs, preferentially utilize this pathway [51]. Because CME is intrinsically coupled to lysosomal trafficking, efficient delivery of degradation-sensitive therapeutics often requires endosomal escape strategies, including pH-responsive materials, proton-sponge polymers or membrane-disruptive peptides, to minimize cargo loss before reaching intracellular targets [52].

4. CAVEOLIN-MEDIATED ENDOCYTOSIS (CVME): MOLECULAR MACHINERY

Caveolin-mediated endocytosis (CvME) is a structurally and mechanistically distinct internalization pathway that depends on cholesterol-rich membrane microdomains and predominantly non-degradative intracellular trafficking. Unlike clathrin-mediated endocytosis, which commonly delivers cargo to lysosomes, CvME utilizes specialized plasma membrane invaginations called caveolae, frequently preserving the integrity of sensitive therapeutic cargo [53]. Coordinated interactions among membrane lipids, scaffolding proteins, and mechanosensitive processes regulate the pathway, making it highly relevant for delivering structurally labile therapeutics intracellularly [54].

4.1 Caveolae architecture: structural basis of CvME

Caveolae are flask-shaped membrane invaginations approximately 50-80 nm in diameter that are enriched in cholesterol, sphingolipids, and caveolins [55]. Caveolin-1, the principal isoform in most non-muscle cells, adopts a hairpin topology and oligomerizes to generate the curved membrane scaffold. Cavin proteins (cavin-1 to cavin-4) reinforce this structure by assembling into a peripheral coat that stabilizes caveolae while allowing dynamic cycles of invagination and flattening in response to cellular conditions. [56,57].

4.2 Lipid dependence and membrane microdomain organization

CvME critically depends on membrane lipid composition. Cholesterol maintains caveolar integrity by stabilizing liquid-ordered membrane domains, whereas cholesterol depletion disrupts caveolae [58]. Sphingolipids further promote ordered, tightly packed domains that recruit proteins such as glycosylphosphatidylinositol (GPI)-anchored receptors and specific integrins, creating favorable platforms for receptor clustering and caveolar uptake [59].

4.3 Mechanism of internalization

Ligand-induced receptor clustering within caveolar domains triggers caveolar invagination and vesicle formation. Membrane scission is frequently mediated by the GTPase dynamin, although its contribution varies with cellular context [60]. Actin polymerization supplies mechanical forces required for vesicle detachment and intracellular transport. Because caveolae are highly mechanosensitive, increased membrane tension flattens them into membrane reservoirs, whereas tension relaxation permits reinvagination, directly linking cellular mechanics to caveolar endocytosis [61].

4.4 Intracellular trafficking and cargo fate

A defining feature of CvME is its non-degradative intracellular trafficking. Caveolar vesicles are frequently delivered to caveosomes, which maintain a near-neutral pH and differ functionally from classical early endosomes [62]. Cargo can subsequently traffic to the endoplasmic reticulum, Golgi apparatus, or undergo transcytosis while largely avoiding lysosomal degradation [63]. Consequently, CvME preserves proteins, peptides, and nucleic acids and improves delivery to intracellular targets that require structural integrity [64].

4.5 Molecular determinants of CvME selectivity

CvME selectivity is governed by membrane lipid composition, receptor organization, and cellular mechanics. Cholesterol- and sphingolipid-rich domains are essential for caveolar stability, while receptors that preferentially partition into these ordered domains, including GPI-anchored proteins and selected integrins, exhibit greater caveolar uptake [65]. Large, highly organized receptor clusters further enhance caveolar recruitment [66]. Membrane tension dynamically regulates caveolar availability through reversible flattening, whereas caveolin-1 expression controls caveolar density and uptake capacity, emphasizing the importance of receptor nano-organization in pathway selection [67].

4.6 Implications for nanomedicine design

CvME provides an attractive route for delivering therapeutics susceptible to lysosomal degradation. Nanoparticles approximately 50-100 nm in diameter that display high ligand valency and target receptors enriched within lipid-ordered domains are more likely to exploit this pathway [68].

Lipid-mimetic or amphiphilic surface modifications can further enhance caveolar recruitment by increasing affinity for cholesterol-rich membranes. Because ligand spacing and surface rigidity strongly influence receptor clustering, optimize these parameters alongside particle size. Nevertheless, CvME remains more cell-type dependent and generally less efficient than CME, so it requires careful validation in heterogeneous tumor systems [69]. Table 1 summarizes the principal molecular distinctions between CME and CvME, including structural proteins, vesicle architecture, lipid composition, receptor specificity, and intracellular trafficking [70]. The table also compares signaling roles, cargo selectivity, and intracellular trafficking routes, providing a concise overview of their functional and mechanistic diversity in cellular uptake processes.

Table 1: Comparative molecular and engineering features of clathrin-mediated versus caveolin-mediated endocytosis

Feature	Clathrin-mediated endocytosis (CME)	Caveolin-mediated endocytosis (CvME)	Engineering Design Intervention	Ref
Vesicle size	~100-150 nm	~50-80 nm	Reduce particle diameter below ~50 nm to favor CME; increase particle size toward ~50-100 nm to promote CvME	[71]
Key structural proteins	Clathrin, AP2, epsin, dynamin	Caveolin-1, cavin, dynamin	Design ligands to selectively recruit AP2-associated receptors or caveolin-associated raft receptors	[72]
Membrane domain	Liquid-disordered regions (PIP ₂ -rich)	Lipid-ordered domains (cholesterol-rich)	Increase cholesterol affinity or incorporate lipid raft-partitioning surface motifs to enhance CvME	[73]
Initiation mechanism	Adaptor-mediated cargo recognition	Lipid raft partitioning and scaffolding	Use receptor-specific ligands containing adaptor-recognition compatibility or raft-targeting architectures	[74]
Curvature generation	Clathrin lattice assembly	Caveolin oligomerization and lipid packing	Engineer nanoparticle curvature and rigidity to match pathway-specific membrane deformation requirements	[75]
Receptor requirements	Cytoplasmic sorting motifs (dileucine)	Raft-associated or GPI-anchored receptors	Select receptors containing canonical internalization motifs for CME or raft-associated receptors for CvME	[76]
Cluster size compatibility	Small, transient clusters (<~50 nm)	Large, stable clusters (>~50-100 nm)	Reduce ligand valency and cluster size for CME; increase multivalent clustering for CvME	[77]
Intracellular routing	Early → late endosome → lysosome	Caveosomes → ER/Golgi/transcytosis	Employ endosomal escape materials for CME or non-degradative trafficking strategies for CvME	[78]
pH exposure	Progressive acidification (pH ~7.4 → ~5)	Near-neutral compartments	Use pH-responsive coatings for CME or neutral-stability formulations for CvME	[79]
Cargo fate	Often degraded	Preserved, potential cytosolic delivery	Use CvME-targeted designs for sensitive biologics, proteins and nucleic acid therapeutics	[80]
Uptake efficiency	High	Moderate, cell-type dependent	Optimize ligand density and receptor accessibility to balance uptake efficiency and pathway selectivity	[81]
Mechanosensitivity	Moderate	High (caveolae flattening response)	Use rigid linker architecture and membrane-active designs to	[82]

Feature	Clathrin-mediated endocytosis (CME)	Caveolin-mediated endocytosis (CvME)	Engineering Design Intervention	Ref
			stabilize caveolar recruitment under mechanical stress	
Ligand spacing	Tight ligand spacing promotes compact adaptor recruitment	Wider ligand spacing favors raft-associated clustering	Increase ligand spacing (>10-20 nm) to promote CvME; tighter spacing favors CME	[77,82]
Ligand valency	Moderate valency supports transient clustering	High valency promotes stable cooperative clustering	Reduce ligand valency for CME; increase multivalent presentation for CvME	[76,77]
Linker flexibility	Flexible linkers support dynamic receptor mobility	Rigid linkers stabilize ordered receptor assemblies	Use rigid linker architecture to stabilize caveolar clustering and ordered membrane organization	[75,82]

5. RECEPTOR NANO-CLUSTERING AS A DETERMINANT OF ENDOCYTIC PATHWAY SELECTION

Receptor nano-clustering is a central mechanistic determinant governing the choice between clathrin-mediated (CME) and caveolin-mediated endocytosis (CvME). Rather than functioning as isolated molecules, cell-surface receptors exist within dynamic membrane environments where their spatial distribution, density and stability regulate downstream trafficking [83]. Multivalent ligand binding, lipid-domain partitioning and cytoskeletal confinement cooperatively generate receptor nanoclusters that serve as platforms for selective recruitment of endocytic machinery, coupling membrane biophysics with intracellular signaling and trafficking [84].

5.1 Cluster size as a threshold parameter

Cluster size is a major biophysical regulator of endocytic pathway selection. Small receptor clusters (<~50 nm) are geometrically compatible with clathrin-coated pits, promoting adaptor recruitment, clathrin lattice assembly and rapid internalization [85]. In contrast, larger clusters (~50-100 nm) preferentially partition into cholesterol-rich lipid rafts and favor caveolar uptake [86]. Membrane-wrapping simulations show that increasing cluster size alters membrane-bending energetics and curvature distribution, shifting the energetic preference from clathrin-supported invagination toward caveolin-stabilized membrane deformation [87]. Super-resolution imaging and computational studies further show that larger, more stable clusters exhibit prolonged membrane residence, enhanced raft partitioning and increased caveolin-1 recruitment, collectively promoting caveola formation and non-degradative trafficking

[88]. Thus, receptor cluster size quantitatively links membrane mechanics with endocytic pathway selection [86-88].

5.2 Spatial organization and ligand-induced geometry

The geometry of receptor clusters strongly influences membrane curvature and endocytic machinery recruitment. Isotropic, densely packed receptor assemblies generate membrane stress patterns favorable for caveolar invagination, particularly within lipid-ordered domains, whereas dispersed or elongated clusters support sequential assembly of clathrin adaptor complexes [87]. Consequently, nanoparticle ligand spacing and surface geometry directly regulate receptor organization and pathway selection [88].

5.3 Cluster density and multivalency

Cluster density is governed by ligand valency and binding avidity. High-valency nanoparticles simultaneously engage multiple receptors, producing cooperative crosslinking that lowers the energetic barrier for membrane deformation and promotes caveolin-associated uptake [89]. In contrast, low-valency interactions generate smaller, sparsely populated clusters that are more compatible with adaptor-mediated CME. Because the relationship between ligand density and clustering is nonlinear, modest increases in valency beyond a critical threshold can produce abrupt shifts in cluster stability and pathway preference [90].

5.4 Temporal stability of clusters

Cluster lifetime also determines endocytic routing. Short-lived receptor clusters preferentially support CME by providing sufficient time for adaptor recruitment and pit nucleation without

extensive membrane reorganization [91]. Persistent clusters instead facilitate cholesterol enrichment and recruitment of caveolin-1 and cavin, promoting caveolar invagination and non-degradative trafficking [92]. Ligand-receptor kinetics, multivalency, and membrane mechanical properties primarily control their stability [93].

5.5 Mechanotransduction and membrane tension

Receptor nano-clustering is tightly coupled to membrane mechanics. Cluster formation generates local membrane deformation, while membrane tension determines the energetic cost of curvature generation [94]. Low membrane tension facilitates both CME and CvME, whereas elevated tension preferentially favors caveolae, which buffer mechanical stress

through reversible flattening and reinvagination [95]. Actin dynamics further regulate receptor mobility, cluster stabilization and coupling of receptor assemblies to specific endocytic pathways, thereby integrating mechanical and biochemical regulation [96].

This Figure 2 presents a multidimensional nanoparticle design framework linking physicochemical properties with endocytic pathway preference. Small, soft, hydrophilic nanoparticles favour clathrin-mediated uptake, whereas larger, rigid, anisotropic particles with ordered ligands and lipid raft affinity preferentially undergo caveolin-mediated endocytosis and non-degradative intracellular trafficking.

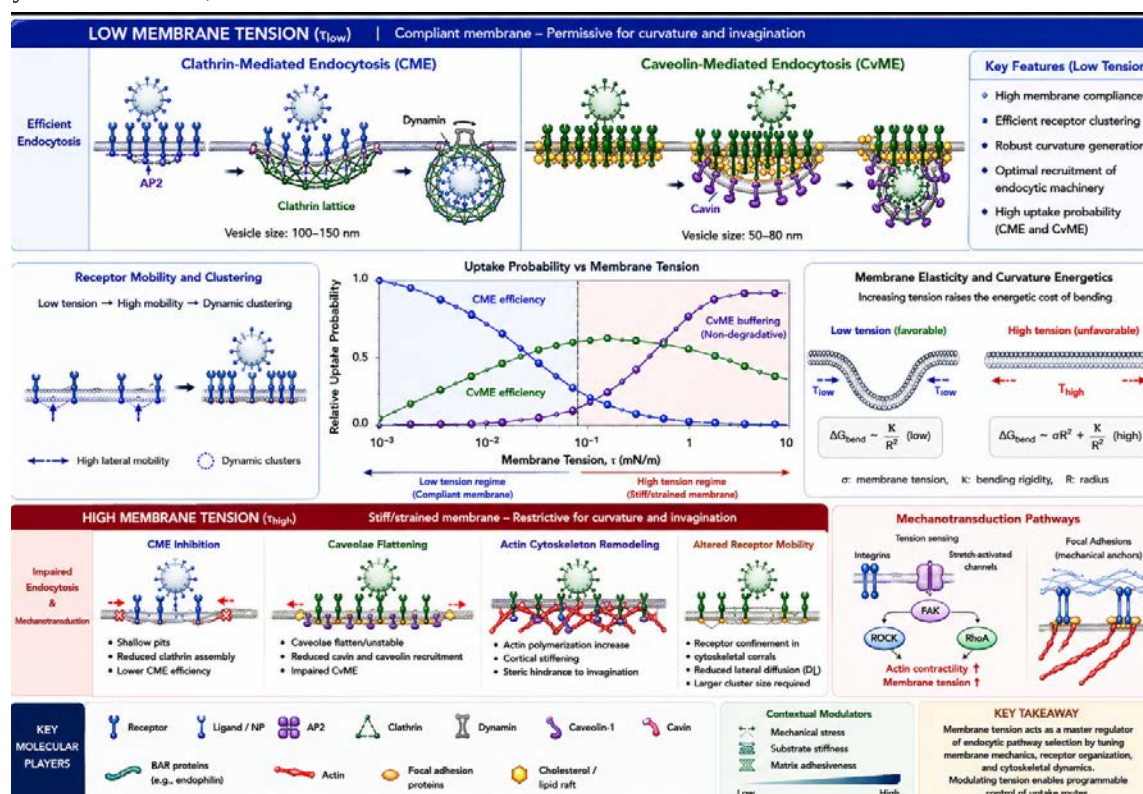


Figure 2: Nanoparticle design rules controlling cellular uptake pathways

(Multidimensional design framework illustrating how nanoparticle physicochemical properties influence endocytic pathway preference. Small, spherical, soft, hydrophilic nanoparticles with flexible ligand spacing preferentially undergo CME, whereas larger, rigid, anisotropic nanoparticles with high ligand density, ordered ligand organization, and lipid raft affinity favor CvME. The figure integrates membrane-wrapping behavior, receptor-spacing geometry, curvature compatibility, and intracellular trafficking outcomes to show how rational nanoparticle engineering can program cellular uptake pathways and therapeutic fate)

5.6 Coupling between nano-clustering and endocytic machinery recruitment

The primary consequence of receptor nano-clustering is the selective recruitment of endocytic machinery. Clusters enriched

in receptors bearing canonical internalization motifs within phosphoinositide-rich membrane regions preferentially recruit AP2 and initiate clathrin-coated pit formation. In contrast, densely packed, long-lived clusters localized in cholesterol-rich

lipid domains favor interaction with caveolin and cavin scaffolds, promoting caveolar internalization [97]. This coupling is cooperative and threshold-dependent: once receptor clusters reach a critical size, density, or stability, recruiting one endocytic machinery suppresses competing pathways, effectively committing the membrane to a specific internalization route [98].

5.7 Implications for rational nanomedicine design

Recognizing receptor nano-clustering as a key determinant of pathway selection provides a predictive framework for nanomedicine design. Engineering nanoparticle size, shape, ligand density and ligand spacing enables controlled formation of receptor clusters with defined structural and dynamic properties [99]. Nanoparticles that generate small, transient clusters with moderate ligand density preferentially promote clathrin-mediated uptake, whereas those inducing large, stable and densely packed clusters within lipid-ordered domains favor caveolin-mediated internalization. Because membrane composition and receptor expression vary across cell types, these design strategies must be optimized for the specific biological context [100].

6. NANOPARTICLE DESIGN PARAMETERS CONTROLLING RECEPTOR NANO-CLUSTERING

Nanoparticles regulate receptor nano-clustering and, in turn, direct endocytic pathway selection through their physicochemical properties. Parameters including size, shape, surface chemistry, ligand density and nanoscale ligand organization collectively control receptor engagement, membrane deformation and cluster stability [101]. These variables modulate the balance between binding energetics, membrane mechanics and receptor mobility, providing a mechanistic basis for engineering programmable cellular entry pathways [102].

6.1 Nanoparticle size: geometric compatibility with membrane curvature

Nanoparticle size is a primary determinant of receptor clustering and membrane wrapping. Particles smaller than ~50 nm generally induce localized receptor engagement and small clusters compatible with clathrin-coated pits, enabling efficient clathrin-mediated uptake [103]. Particles of ~50-100 nm promote larger receptor assemblies and broader membrane deformation that favor cholesterol-rich caveolar domains, whereas particles exceeding ~150 nm require more membrane-bending energy and often engage alternative uptake mechanisms

such as macropinocytosis [104]. These effects reflect the relationship between nanoparticle curvature and membrane bending rigidity, with smaller particles matching clathrin geometry and larger particles favoring caveolar membrane curvature [105].

6.2 Nanoparticle shape: anisotropy and curvature distribution

Nanoparticle shape regulates receptor organization by altering membrane contact geometry. Spherical nanoparticles produce relatively uniform receptor engagement, whereas anisotropic particles such as rods, ellipsoids, and discs generate directional receptor assemblies and heterogeneous membrane curvature [106]. High-aspect-ratio nanorods redistribute membrane stress and curvature-dependent protein binding, while disc-like particles promote extended receptor clusters that are less compatible with CME and more likely to associate with lipid-ordered domains [107,108].

6.3 Surface ligand density: regulation of multivalent interactions

Ligand density directly controls receptor crosslinking and cluster formation. Low ligand density favors monovalent interactions and small receptor clusters characteristic of CME [109]. Increasing ligand density promotes multivalent binding, cooperative receptor clustering, and reduced energetic barriers for membrane deformation, thereby enhancing caveolar recruitment [110]. Because clustering is highly cooperative, exceeding a critical ligand-density threshold produces disproportionate increases in cluster size and stability [111].

6.4 Spatial patterning of ligands: nanoscale organization

The nanoscale arrangement of ligands determines receptor spacing and cluster geometry. Random ligand distribution produces stochastic receptor engagement, whereas precisely patterned ligands optimize receptor crosslinking and cooperative interactions [112]. Nanofabrication approaches such as DNA scaffolding and template-directed assembly enable sub-nanometre control of ligand spacing, allowing receptor organizations that selectively favor specific endocytic pathways [113].

6.5 Ligand affinity and avidity: thermodynamic and kinetic control

Nanoparticle binding depends on both ligand affinity and multivalent avidity. High-affinity ligands alone may not

generate stable receptor clusters, whereas multivalent interactions markedly increase effective binding strength, prolong receptor residence time, and stabilize receptor assemblies [114]. This enhanced avidity promotes membrane reorganization and recruitment of endocytic machinery.

The balance between affinity, avidity, and binding kinetics ultimately determines whether receptor clusters preferentially engage CME or CvME [115,116].

6.6 Surface chemistry and physicochemical properties

Surface chemistry further modulates receptor clustering. Polyethylene glycol (PEG) improves colloidal stability and reduces nonspecific interactions, although excessive PEG density can hinder ligand accessibility [117]. Surface charge also influences uptake: highly positive nanoparticles promote nonspecific membrane interactions, whereas neutral or mildly negative surfaces better preserve receptor-specific clustering. A protein corona can also mask targeting ligands or introduce new receptor interactions, substantially altering clustering behavior in vivo [118,119].

6.7 Integrated design considerations

Nanoparticle design parameters act cooperatively rather than independently. Ligand density effects depend on particle size, curvature, ligand spacing, and surface chemistry, requiring simultaneous optimization of multiple physicochemical variables [120].

Integration of predictive modeling with experimental validation is therefore essential for identifying nanoparticle designs that produce desired receptor-clustering behavior and selective endocytic pathway engagement. Table 2 summarizes how nanoparticle size, shape, surface chemistry, ligand density, and ligand organization influence receptor nano-clustering and subsequent pathway selection [121]. Figure 3 also highlights how surface chemistry and elasticity modulate cellular internalization routes, signaling activation, and intracellular trafficking. It provides a concise framework linking nanoparticle physicochemical properties to targeted uptake mechanisms and pathway bias

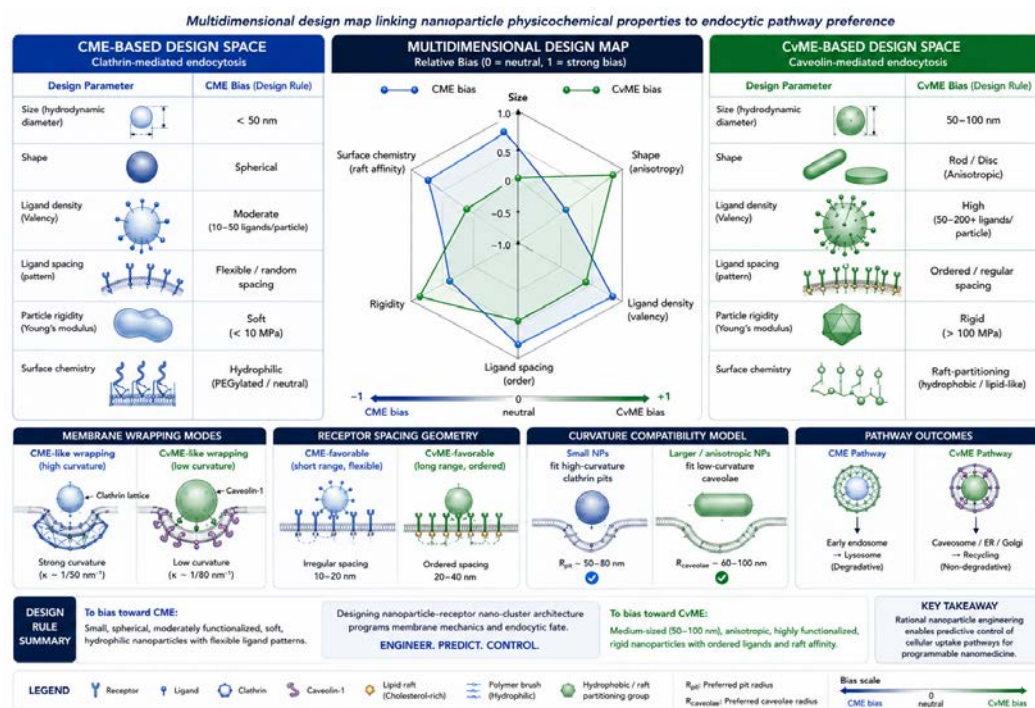


Figure 3: Mechanobiology of membrane tension and endocytic switching

(Multidimensional design framework illustrating how nanoparticle physicochemical properties influence endocytic pathway preference. Small, spherical, soft, hydrophilic nanoparticles with flexible ligand spacing preferentially undergo CME, whereas larger, rigid, anisotropic nanoparticles with high ligand density, ordered ligand organization, and lipid raft affinity favor CvME. The figure integrates membrane-wrapping behavior, receptor-spacing geometry, curvature compatibility, and intracellular trafficking outcomes to show how rational nanoparticle engineering can program cellular uptake pathways and therapeutic fate)

Table 2: Nanoparticle design parameters governing receptor nano-clustering and endocytic pathway bias

Design parameter	Molecular effect on receptor clustering	Bias toward CME	Bias toward CvME	Ref.
Particle size	Determines membrane curvature compatibility and receptor contact area	<50 nm	50-100 nm	[122]
Shape	Regulates spatial distribution of interaction forces at the membrane interface	Spherical, isotropic	Rod-like or disc-shaped, anisotropic	[123]
Ligand density	Controls multivalency and extent of receptor crosslinking	Low to moderate	High	[124]
Ligand spacing	Governs inter-receptor distance and cooperative binding interactions	Random or flexible spacing	Precisely defined and ordered spacing	[125]
Ligand affinity	Influences binding kinetics and stability of receptor engagement	High affinity with low avidity	Moderate affinity with high avidity	[126]
Surface charge	Modulates nonspecific electrostatic interactions with the membrane	Neutral or slightly negative	Slightly negative with affinity for lipid raft domains	[127]
Surface chemistry	Determines membrane partitioning and lipid domain association	Hydrophilic, compatible with PIP ₂ -rich regions	Cholesterol-mimetic or raft-partitioning surfaces	[128]
Linker flexibility	Regulates receptor mobility and conformational adaptability within clusters	Flexible linkers	Rigid linkers	[129]
Mechanical rigidity	Influences membrane deformation and wrapping efficiency	Structurally flexible nanoparticles	Structurally rigid nanoparticles	[130]
Protein corona	Alters ligand accessibility and effective surface identity in biological environments	Minimal masking of targeting ligands	Controlled corona formation enhancing raft interactions	[131]

7. MOLECULAR DESIGN RULES FOR BIASING CLATHRIN VERSUS CAVEOLIN-MEDIATED UPTAKE

Selective engagement of clathrin-mediated endocytosis (CME) or caveolin-mediated endocytosis (CvME) is a key objective in rational nanomedicine design. Although receptor targeting determines cellular specificity, the nanoscale organization of receptor engagement ultimately governs intracellular trafficking. Effective molecular design therefore integrates membrane biophysics, receptor nano-clustering and nanoparticle architecture to achieve predictable pathway bias [132].

7.1 Design principles favoring clathrin-mediated endocytosis

Nanoparticles intended for CME should promote small, transient receptor clusters compatible with AP2 recruitment and clathrin-

coated pit assembly [133]. Particles approximately 20-50 nm in diameter closely match clathrin curvature, minimizing membrane deformation energy. Moderate ligand density limits excessive receptor crosslinking while maintaining dynamic clustering required for adaptor recognition [134]. Targeting ligands should engage receptors bearing canonical tyrosine-based or dileucine internalization motifs recognized by AP2 [135]. Flexible ligand linkers and targeting of receptors within phosphoinositide-rich, liquid-disordered membrane regions further support efficient clathrin-mediated uptake [134].

7.2 Design principles favoring caveolin-mediated endocytosis

CvME requires nanoparticles that generate large, stable receptor clusters within lipid-ordered membrane domains. Particle sizes of approximately 50-100 nm provide membrane contact areas

compatible with caveolae [135]. High ligand valency promotes extensive receptor crosslinking and stable cluster formation that recruits caveolin scaffolds. Target receptors should preferentially partition into cholesterol-rich domains, including glycosylphosphatidylinositol (GPI)-anchored proteins and selected integrins. Rigid ligand presentation, together with lipid-mimetic or amphiphilic surface modifications, enhances receptor organization within caveolar domains, favoring non-degradative intracellular trafficking and preserving sensitive therapeutic cargo [136].

7.3 Modulation of ligand affinity and binding kinetics

Ligand affinity, avidity, and binding kinetics critically regulate pathway selection. Moderate-affinity ligands presented multivalently generate stable receptor clusters that favor CvME, whereas high-affinity monovalent interactions better match the dynamic receptor turnover characteristic of CME. Association and dissociation rates determine cluster lifetime, with persistent clusters promoting caveolin recruitment and transient assemblies favoring clathrin adaptor binding [137].

7.4 Influence of membrane composition and cellular context

The success of pathway-specific nanoparticle design depends on membrane composition and receptor expression. Cells enriched in cholesterol, lipid-ordered domains, and caveolin-1 are more favorable for CvME, whereas membranes enriched in phosphoinositides and active clathrin machinery preferentially support CME. Consequently, nanoparticle design must be adapted to the membrane properties and receptor landscape of the target cell, particularly in heterogeneous tumors [138].

7.5 Hybrid and programmable design strategies

Emerging nanomedicines increasingly use programmable systems that dynamically regulate receptor clustering and endocytic pathway selection. Stimuli-responsive nanoparticles, for example, can minimize ligand exposure during circulation and activate multivalent receptor clustering within the tumor microenvironment to enhance caveolar uptake. Sequential targeting strategies that combine initial CME with subsequent intracellular transformations for endosomal escape further illustrate the potential of adaptive nanoparticle design [139].

7.6 Trade-offs and design constraints

Pathway-selective design requires balancing efficiency with intracellular fate. CME enables rapid, efficient uptake but is

intrinsically linked to degradative trafficking, whereas CvME better preserves therapeutic cargo but is generally more cell-type dependent and less efficient. Excessive receptor clustering may also trigger unintended signaling, receptor downregulation, or altered pharmacokinetics. Successful nanoparticle design therefore requires optimization of pathway bias alongside these biological constraints [140].

8. RECEPTOR-SPECIFIC DETERMINANTS AND CASE STUDIES IN PATHWAY BIAS

Although general nanoparticle design principles guide endocytic pathway selection, the target receptor's intrinsic properties ultimately determine the internalization route. Differences in receptor structure, membrane localization, clustering dynamics, and coupling to intracellular machinery define how nanoparticle-induced nano-clustering influences clathrin-mediated endocytosis (CME) or caveolin-mediated endocytosis (CvME). Understanding these receptor-specific characteristics is therefore essential for translating design principles into predictable intracellular trafficking outcomes [141].

8.1 Epidermal growth factor receptor: clustering-dependent modulation of clathrin-mediated endocytosis

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that undergoes ligand-induced dimerization followed by higher-order clustering. Because of its canonical internalization motifs and recruitment of AP2 and epsin, EGFR is internalized predominantly through CME [142]. Moderate ligand valency promotes transient receptor clusters compatible with clathrin-coated pits, whereas excessive multivalent crosslinking may alter receptor organization and trafficking. Consequently, EGFR-targeted nanoparticles require careful optimization of ligand density and spacing to preserve efficient clathrin-mediated uptake [142].

8.2 Integrins: mechanosensitive clustering and caveolar coupling

Integrins are mechanosensitive receptors that interact extensively with the actin cytoskeleton and membrane microdomains. Unlike receptor tyrosine kinases, they exhibit context-dependent internalization through both CME and CvME [143]. Multivalent nanoparticle binding promotes large, mechanically stable receptor clusters that preferentially partition into cholesterol-rich lipid domains and recruit caveolin, particularly under conditions of high ligand density and mechanical stress. Strong multivalent interactions therefore

enhance caveolar uptake of integrin-targeted nanoparticles [144].

8.3 Transferrin receptor: canonical clathrin-dependent internalization

The transferrin receptor is a classical model of CME, undergoing constitutive internalization through adaptor-recognized sorting

motifs while continuously recycling to maintain iron homeostasis [145]. Transferrin-functionalized nanoparticles generally induce moderate receptor clustering compatible with clathrin-coated pits. Excessive ligand valency or rigid ligand presentation, however, can disrupt physiological clustering and alter receptor recycling, emphasizing the need to preserve the receptor's native organization [145].

Table 3. Receptor-specific clustering behavior and preferred endocytic pathways

Receptor	Membrane localization	Clustering behaviour	Preferred endocytic pathway	Design implications for nanomedicine	Ref
Epidermal growth factor receptor (EGFR)	Primarily non-raft; can redistribute upon activation	Ligand-induced dimerization followed by transient oligomerization	Predominantly clathrin-mediated endocytosis	Maintain moderate ligand density to avoid excessive clustering and preserve CME	[150]
Human epidermal growth factor receptor 2 (HER2)	Mixed membrane domains	Stable, overexpression-driven clustering	Clathrin-mediated dominant	Control multivalency to prevent aberrant receptor aggregation	[151]
Integrins (e.g. $\alpha\beta3$, $\alpha5\beta1$)	Lipid raft-associated; coupled to cytoskeleton	Mechanosensitive, large and stable clusters	Caveolin-mediated under multivalent conditions	Employ high ligand valency and controlled spatial presentation	[152]
Transferrin receptor (TfR)	Non-raft regions	Constitutive small clusters with rapid turnover	Clathrin-mediated endocytosis	Ideal model for CME targeting; avoid excessive crosslinking	[153]
Folate receptor (GPI-anchored)	Lipid raft domains	Dense clustering confined to cholesterol-rich regions	Caveolin-mediated endocytosis	Highly suitable for caveolar targeting via multivalent ligand presentation	[154]
Scavenger receptors	Mixed membrane distribution	Large multivalent assemblies upon ligand binding	Mixed or non-clathrin pathways	Minimize nonspecific interactions through controlled surface design	[155]
G-Protein coupled receptors (GPCRs)	Variable; context-dependent	Dynamic and transient clustering behavior	Context-dependent (CME or non-clathrin)	Tune ligand affinity and kinetics to modulate pathway selection	[156]

8.4 Folate receptor: lipid raft localization and caveolin-mediated uptake

The folate receptor is a glycosylphosphatidylinositol (GPI)-anchored protein localized almost exclusively within lipid-ordered membrane domains, predisposing it to CvME [146]. Multivalent folate-functionalized nanoparticles induce dense receptor clusters that remain within cholesterol-rich domains and efficiently recruit caveolar machinery. Because the receptor

lacks a cytoplasmic domain, coupling to endocytosis depends primarily on its lipid environment, making it particularly suitable for caveolin-targeted delivery strategies [146].

8.5 Scavenger receptors and pattern recognition receptors

Scavenger receptors exhibit broad ligand specificity and internalize nanoparticles through both clathrin-dependent and independent pathways. Ligand binding frequently induces large

multimeric receptor assemblies, especially on multivalent or negatively charged nanoparticle surfaces [147]. Extensive receptor crosslinking can favor caveolar or alternative non-clathrin pathways, although broad ligand recognition also increases the risk of nonspecific uptake, necessitating careful surface engineering [147].

8.6 Receptor expression levels and saturation effects

Receptor abundance strongly influences clustering dynamics and pathway selection. High receptor expression enhances multivalent engagement and formation of large receptor clusters that may favor CvME, whereas low receptor density limits cluster size and generally supports CME. Excessive nanoparticle binding can saturate receptor populations, altering normal trafficking and promoting alternative uptake pathways [148].

8.7 Interplay between receptor signaling and endocytosis

Receptor nano-clustering regulates both endocytosis and intracellular signaling. EGFR clustering enhances kinase activation, whereas integrin clustering activates mechanotransduction pathways that influence adaptor recruitment, phosphorylation and cytoskeletal remodeling [149]. Because these signaling events feed back to regulate endocytic machinery, nanoparticle-induced receptor clustering must be carefully controlled to avoid unintended biological responses. Table 3 summarizes receptor-specific clustering behaviors, adaptor recruitment, membrane localization and preferred endocytic pathways, providing a mechanistic framework for receptor-guided nanocarrier design [149].

9. INTRACELLULAR TRAFFICKING CONSEQUENCES OF ENDOCYTIC PATHWAY SELECTION

The intracellular fate of nanoparticle-delivered therapeutics depends not only on successful cellular internalization but, more critically, on the trafficking pathways engaged thereafter. Clathrin-mediated and caveolin-mediated endocytosis direct cargo through fundamentally distinct intracellular compartments, each characterized by specific physicochemical environments, enzymatic activities, and transport dynamics. These differences profoundly affect the stability, bioavailability, and ultimate therapeutic efficacy of delivered agents [157]. Nanoparticles internalized via clathrin-mediated pathways typically traffic toward acidic endolysosomal compartments, promoting degradation or controlled drug release, whereas caveolin-mediated uptake bypasses lysosomes and enables cytosolic or organelle-specific delivery. These distinct

intracellular trafficking routes critically influence therapeutic stability, bioavailability, subcellular localization, and overall nanomedicine efficacy.

9.1 Endosomal maturation and trafficking dynamics in clathrin-mediated uptake

Following clathrin-mediated internalization, vesicles rapidly uncoat and fuse with early endosomes, the primary sorting compartments of the endocytic network. Early endosomes (pH ~6.0-6.5), characterized by Rab5 and a dynamic tubulovesicular architecture, direct cargo either to recycling pathways or toward late endosomes. Maturation involves Rab5-to-Rab7 conversion, membrane remodeling, and progressive acidification. Late endosomes (pH ~5.0-5.5) subsequently fuse with lysosomes (pH ~4.5-5.0), where vacuolar-type H⁺-ATPases establish an acidic environment that activates hydrolytic enzymes, including proteases, nucleases, and lipases, leading to cargo degradation [158].

9.2 Endosomal escape: a critical barrier in clathrin-mediated delivery

For therapeutics requiring cytosolic or nuclear access, endosomal escape is essential because a large fraction of internalized cargo is otherwise degraded in lysosomes. Strategies to promote escape include the proton sponge effect of cationic polymers such as polyethyleneimine, as well as pH-responsive lipids and membrane-active peptides that disrupt endosomal membranes under acidic conditions [159]. Efficient escape requires balancing membrane destabilization with preservation of cellular viability [160].

9.3 Lysosomal degradation and therapeutic stability

Lysosomes are the terminal degradative compartment of CME and contain hydrolytic enzymes active under acidic conditions that efficiently degrade proteins, nucleic acids, and lipid-based therapeutics [161]. While beneficial for lysosomotropic prodrugs, this environment limits delivery of structurally sensitive biomolecules, making lysosomal avoidance or early endosomal escape essential for cytosolic therapies.

9.4 Caveolin-mediated trafficking: non-degradative pathways

In contrast, caveolin-mediated endocytosis frequently bypasses lysosomal degradation. Caveolar vesicles traffic to caveosomes, which maintain near-neutral pH and low degradative activity, before transporting cargo to the endoplasmic reticulum, Golgi

apparatus or through transcytosis [162]. Distinct Rab GTPases, lipid composition, and cytoskeletal interactions differentiate this pathway from classical endosomal trafficking, preserving sensitive therapeutic cargo.

9.5 Cytosolic delivery and organelle targeting

CvME is particularly advantageous for cytosolic delivery of gene-editing systems, nucleic acids, and protein therapeutics because it minimizes exposure to degradative compartments. Targeted trafficking to organelles such as the endoplasmic reticulum or mitochondria further expands therapeutic

opportunities, including modulation of protein processing, metabolism and apoptosis [163].

9.6 Interplay between trafficking and cellular signaling

Intracellular trafficking is closely linked to cellular signaling. Endosomes function as signaling platforms, particularly for receptor tyrosine kinases, with CME often supporting sustained endosomal signaling [164]. CvME can modify or attenuate signaling depending on receptor type and cellular context, indicating that nanoparticle-directed trafficking may influence cellular responses beyond cargo delivery.

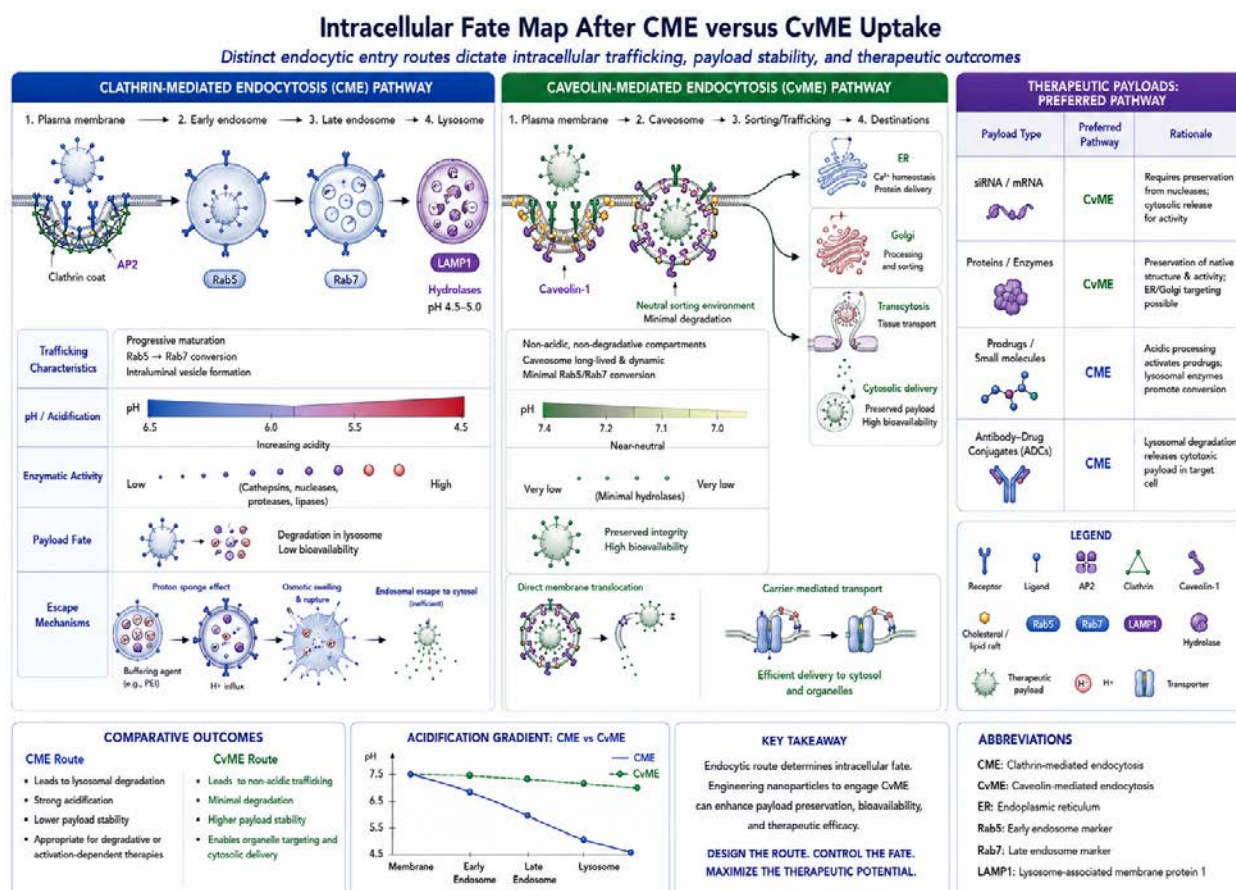


Figure 4: Intracellular fate map after CME versus CvME uptake
(Schematic representation of intracellular trafficking pathways following nanoparticle internalization via clathrin-mediated and caveolin-mediated endocytosis. The diagram highlights differential routing through endosomes, lysosomes, or caveosomes, illustrating their influence on cargo degradation, intracellular transport, subcellular targeting, and the therapeutic efficacy of nanomedicine-based delivery systems)

9.7 Implications for rational nanomedicine design

The selected endocytic pathway should match the therapeutic payload's desired intracellular destination. CME is advantageous for rapid uptake and applications benefiting from lysosomal processing, whereas CvME is preferred for preserving labile biomolecules and enabling cytosolic delivery. Rational

nanoparticle design should therefore integrate uptake mechanism, intracellular trafficking, and target localization by incorporating endosomal escape strategies for CME or enhancing caveolar targeting through receptor selection and nanoparticle engineering [165].

10. EXPERIMENTAL AND ANALYTICAL TECHNIQUES TO RESOLVE RECEPTOR NANO-CLUSTERING AND ENDOCYTIC PATHWAY SELECTION

Understanding receptor nano-clustering and its relationship to endocytic pathway selection requires experimental methods that can resolve molecular events from individual receptor interactions to whole-cell trafficking. Conventional biochemical assays lack the spatial and temporal resolution necessary for this purpose. Consequently, advanced imaging, quantitative biophysical methods, and computational modeling have become essential for correlating receptor organization with membrane dynamics and intracellular trafficking [166].

10.1 Super-resolution microscopy: direct visualization of nanoclusters

Super-resolution techniques such as stochastic optical reconstruction microscopy (STORM) and photoactivated localization microscopy (PALM) overcome the diffraction limit, achieving resolutions of approximately 10-30 nm for direct visualization of receptor nanoclusters [167].

These methods quantify cluster size, density and spatial organization while enabling colocalization with clathrin, caveolin-1 and adaptor proteins. Live-cell imaging further reveals the dynamics of cluster formation, growth and disassembly.

10.2 Single-particle tracking: dynamics of receptor mobility

Single-particle tracking (SPT) follows individual receptors or nanoparticles in real time, providing diffusion coefficients, confinement behavior and transition states [168]. Changes in receptor mobility following nanoparticle binding reveal cluster formation, cytoskeletal confinement and membrane-domain association. Characteristic transitions from free diffusion to confined motion also identify endocytic events and help distinguish CME from CvME.

10.3 Förster resonance energy transfer: nanoscale proximity measurements

Forster resonance energy transfer (FRET) measures intermolecular distances of approximately 1-10 nm, making it highly sensitive to receptor clustering [169].

Increased FRET efficiency reflects closer receptor proximity, while fluorescence lifetime imaging microscopy (FLIM-FRET) provides concentration-independent quantification of clustering kinetics and nanoparticle-induced receptor organization.

10.4 Electron microscopy: ultrastructural characterization

Transmission electron microscopy (TEM), scanning electron microscopy (SEM) and cryo-electron microscopy provide nanometre-scale visualization of membrane architecture, clathrin-coated pits and caveolae [170]. Immunogold labeling further localizes receptors or nanoparticles within endocytic structures, validating receptor clustering and vesicle morphology observed by optical imaging.

10.5 Proteomic and biochemical analysis of endocytic vesicles

Subcellular fractionation combined with mass spectrometry-based proteomics enables characterization of clathrin-coated vesicles, caveolar fractions and endosomal compartments [171]. Quantitative proteomics identifies pathway-specific protein signatures, whereas immunoblotting and co-immunoprecipitation reveal recruitment of adaptor proteins and trafficking regulators to receptor complexes.

10.6 Fluorescence-based trafficking assays

Fluorescence microscopy using compartment-specific markers and pH-sensitive probes tracks intracellular cargo trafficking and distinguishes caveolar from endo-lysosomal routing [172]. Time-lapse colocalization with early endosomes, late endosomes, lysosomes, and caveolar markers enables quantitative analysis of intracellular transport kinetics and nanoparticle fate.

10.7 Computational modeling and quantitative analysis

Computational approaches complement experimental studies by predicting receptor clustering and endocytic pathway selection. Molecular dynamics simulations describe ligand-receptor interactions, continuum membrane models quantify curvature generation and agent-based models simulate receptor diffusion and clustering kinetics [173]. Machine learning further integrates multidimensional datasets to predict uptake pathways from nanoparticle design parameters.

10.8 Integration of multimodal approaches

A comprehensive understanding of receptor nano-clustering requires integration of complementary methodologies. Super-resolution microscopy defines spatial organization, SPT captures receptor dynamics, FRET measures molecular proximity, electron microscopy validates ultrastructure and biochemical analyses identify molecular composition [174].

Together, these multimodal approaches establish mechanistic links between nanoparticle design, receptor organization, endocytic machinery recruitment, intracellular trafficking and therapeutic outcome.

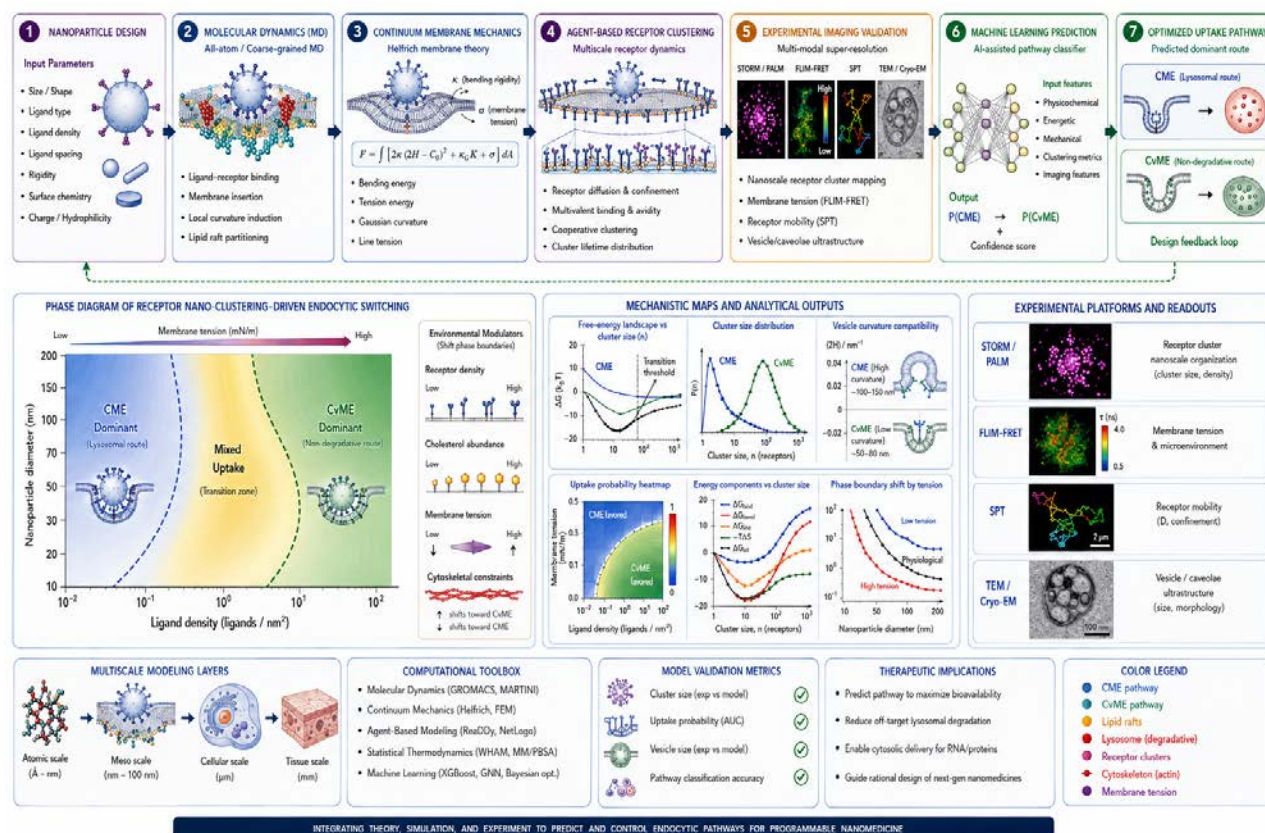


Figure 5: Integrated predictive computational-experimental framework

(Illustration of integrated multimodal approaches used to investigate receptor nano-clustering and endocytic pathway selection. The diagram combines super-resolution microscopy, single-particle tracking, FRET analysis and biochemical assays to correlate nanoparticle design with receptor organization, uptake mechanisms, intracellular trafficking and therapeutic performance)

11. COMPUTATIONAL AND THEORETICAL FRAMEWORKS FOR PREDICTING RECEPTOR NANO-CLUSTERING AND ENDOCYTIC PATHWAY SELECTION

The complexity of receptor nano-clustering and endocytic pathway selection requires computational approaches that complement experimental observations by resolving interactions across multiple spatial and temporal scales. These models systematically explore the effects of nanoparticle design, membrane composition, and receptor dynamics, providing quantitative and predictive frameworks for nanoparticle-cell interactions [175].

11.1 Molecular dynamics simulations: atomistic and coarse-grained approaches

Molecular dynamics (MD) simulations provide mechanistic insight into nanoparticle interactions with lipid membranes and

receptors. Atomistic MD resolves ligand-receptor binding, conformational changes, and local membrane perturbations but is limited in simulation scale [176]. Coarse-grained models, including the Martini force field, extend simulations to larger systems and longer times, enabling investigation of membrane deformation, receptor clustering and early vesicle formation [177]. These simulations generate quantitative estimates of binding energies, diffusion coefficients and membrane mechanical properties that govern receptor clustering.

11.2 Continuum models of membrane mechanics

Continuum models describe the membrane as an elastic surface defined by bending rigidity, membrane tension and spontaneous curvature. Using the Helfrich energy formalism, these models quantify the energetic cost of nanoparticle wrapping and membrane deformation [178]. Integrating receptor-binding

energetics with membrane mechanics predicts whether internalization is favorable and whether CME or CvME is energetically preferred.

11.3 Statistical thermodynamics of receptor clustering

Statistical thermodynamic models describe receptor clustering by balancing ligand-receptor binding energy with entropic effects arising from receptor diffusion and confinement [179]. Lattice and mean-field approaches predict cluster formation as a function of receptor density and ligand valency, revealing cooperative transitions from dispersed receptors to stable nanoclusters that can recruit endocytic machinery.

11.4 Agent-based and mesoscopic models

Agent-based models simulate receptors, lipids and nanoparticles as interacting entities undergoing diffusion, binding and clustering [180]. These stochastic simulations reproduce cluster nucleation, growth, coalescence and coupling to endocytic structures, while mesoscopic models bridge molecular-scale events with cellular membrane dynamics.

11.5 Machine learning and data-driven prediction

Machine learning identifies complex relationships between nanoparticle properties and endocytic behavior. Supervised models classify likely uptake pathways, whereas regression and deep learning approaches integrate imaging, proteomic and physicochemical datasets to predict uptake efficiency, intracellular trafficking and pathway selection [181]. These data-driven methods complement mechanistic models by rapidly exploring high-dimensional design spaces.

11.6 Multiscale modeling: integration across hierarchical levels

Multiscale frameworks integrate molecular simulations, continuum membrane mechanics, and cellular-scale models to describe receptor clustering from ligand binding to intracellular trafficking [182]. Parameters derived from molecular simulations inform mesoscale models of cluster formation and vesicle dynamics, enabling prediction of how nanoparticle design influences cellular responses and therapeutic performance.

11.7 Model validation and experimental integration

Reliable computational prediction requires validation against experimental measurements obtained from super-resolution microscopy, single-particle tracking, and biochemical analyses

[183]. Iterative integration of modeling and experimentation refines model parameters, identifies critical determinants of receptor clustering and endocytic pathway selection, and supports the rational optimization of nanoparticle design.

12. TUMOR MICROENVIRONMENTAL MODULATION OF RECEPTOR NANO-CLUSTERING AND ENDOCYTIC PATHWAYS

The tumor microenvironment profoundly influences plasma membrane organization, receptor nano-clustering and endocytic pathway selection. Unlike normal tissues, tumors exhibit heterogeneous biochemical and biophysical conditions, including altered lipid composition, mechanical stress, hypoxia, acidosis and dysregulated signaling, that reshape receptor organization and nanoparticle uptake. Consequently, nanoparticles optimized in vitro may behave markedly differently in vivo [184].

12.1 Alterations in membrane lipid composition

Tumor cells frequently exhibit increased cholesterol, altered phospholipid saturation and elevated glycosphingolipid content, modifying membrane fluidity, thickness and lipid-domain organization [185].

Cholesterol-rich membranes stabilize lipid rafts and caveolae, promoting receptor partitioning into ordered domains and favoring caveolin-mediated uptake, whereas changes in phosphoinositide distribution influence adaptor recruitment required for CME. These lipid alterations reshape membrane energetics, receptor clustering and endocytic pathway selection [185].

12.2 Receptor expression and glycosylation patterns

Tumor cells commonly overexpress receptor tyrosine kinases, integrins and other surface receptors, increasing opportunities for multivalent nanoparticle engagement and large receptor-cluster formation [186]. Aberrant glycosylation further modifies receptor conformation, membrane partitioning and steric accessibility, thereby regulating receptor nano-clustering and coupling to endocytic machinery.

12.3 Mechanical properties and membrane tension

Tumor-associated stiffness and elevated interstitial pressure increase membrane tension and alter cytoskeletal organization [187]. High membrane tension raises the energetic cost of membrane deformation, limiting CME, whereas caveolae

function as mechanosensitive membrane reservoirs that flatten and reinvaginate to buffer mechanical stress. Actin remodeling further stabilizes receptor clusters and regulates their coupling to specific uptake pathways.

12.4 Hypoxia and metabolic reprogramming

Hypoxia activates hypoxia-inducible factors that regulate genes involved in membrane trafficking, lipid metabolism and endocytosis [188]. These changes alter expression of clathrin, caveolin-1 and associated adaptor proteins while modifying membrane lipid composition. Hypoxia-induced acidosis additionally affects ligand-receptor interactions, nanoparticle stability and intracellular trafficking dynamics.

12.5 Extracellular matrix and cell-matrix interactions

The dense and mechanically rigid tumor extracellular matrix restricts nanoparticle transport while regulating receptor clustering through integrin-mediated adhesion [189]. Strong cell-matrix interactions stabilize integrin clusters and focal adhesions that frequently favor caveolar uptake, whereas matrix composition also influences nanoparticle diffusion and receptor accessibility.

12.6 Protein corona formation in biological environments

Following exposure to biological fluids, nanoparticles rapidly acquire a dynamic protein corona that alters their biological identity by changing hydrodynamic size, surface charge and ligand accessibility [190]. Corona formation can mask targeting ligands, disrupt receptor nano-clustering and redirect nanoparticles toward unintended uptake pathways. Because corona composition continuously evolves with local protein composition, pH and inflammatory conditions, receptor recognition and intracellular trafficking may differ substantially from in vitro predictions [191].

Although PEGylation reduces nonspecific protein adsorption, it does not fully prevent corona formation and may contribute to immune recognition or ligand shielding. Emerging biomimetic strategies-including cell membrane cloaking, zwitterionic coatings, albumin pre-coating and adaptive surface engineering-better preserve receptor accessibility. Complementary approaches such as rigid ligand linkers, increased ligand protrusion, optimized ligand spacing and stimuli-responsive ligand exposure help maintain receptor nano-clustering despite corona formation, improving reliable bias toward CME or CvME in vivo [192].

12.7 Heterogeneity and spatial variability

Tumors exhibit marked spatial heterogeneity in receptor expression, membrane composition, mechanics and metabolism, producing regional differences in endocytic pathway utilization [193]. Areas enriched in caveolin and lipid-ordered domains may preferentially support CvME, whereas others favor CME. This variability highlights the need for adaptable nanoparticle systems that maintain pathway-selective uptake across heterogeneous tumor microenvironments [193].

13. TRANSLATIONAL CONSIDERATIONS IN PATHWAY-SELECTIVE NANOMEDICINE DESIGN

Translation of pathway-selective nanomedicine from controlled in vitro systems to clinical application involves challenges that extend beyond molecular design. Although receptor nano-clustering and endocytic pathway bias can be engineered experimentally, their in vivo performance is strongly influenced by biological variability, systemic physiology and immune interactions. Preserving pathway-selective uptake therefore requires consideration of nanoparticle stability, pharmacokinetics, protein corona formation and physiological barriers [194].

13.1 Protein corona formation and its impact on targeting fidelity

Upon exposure to biological fluids, nanoparticles rapidly acquire a protein corona that alters their effective size, surface charge and ligand accessibility [195]. Corona formation can mask targeting ligands, reduce receptor-specific binding and disrupt receptor nano-clustering while introducing new recognition motifs that redirect nanoparticles toward unintended uptake pathways. Strategies such as surface passivation, optimized ligand orientation and corona-control approaches are therefore essential for maintaining targeting fidelity and pathway selectivity.

13.2 Pharmacokinetics and biodistribution

Nanoparticle pharmacokinetics determine circulation time, biodistribution and clearance. Surface modifications such as polyethylene glycol (PEG) prolong circulation by reducing immune recognition, although excessive shielding can impair receptor engagement and nano-clustering [196]. Nanoparticle size, geometry, and surface properties further influence tumor extravasation, interstitial penetration, and cellular uptake, requiring a careful balance between systemic stability and efficient receptor targeting.

13.3 Immunological interactions and clearance mechanisms

Interactions with macrophages and other immune cells can promote rapid clearance through opsonization, complement activation, and pattern-recognition receptors, reducing nanoparticle accumulation at tumor sites [197]. Surface charge, hydrophobicity, and protein corona composition strongly influence these processes. Because immune cells often internalize nanoparticles through pathways distinct from tumor cells, minimizing unintended immune uptake is essential to preserve pathway specificity.

13.4 Discrepancies between in vitro and in vivo systems

Controlled in vitro models often fail to reproduce the complexity of physiological environments [198]. Differences in membrane composition, receptor expression, tissue mechanics, and extracellular barriers can substantially alter nanoparticle behavior and endocytic pathway selection. Advanced experimental systems, including three-dimensional cultures and animal models, are therefore required to better predict in vivo performance.

13.5 Tumor heterogeneity and patient-specific variability

Tumor heterogeneity in receptor expression, membrane composition and microenvironmental conditions produces considerable variability in receptor clustering, nanoparticle uptake and therapeutic response [199].

Adaptive nanoparticle designs incorporating multiple targeting ligands or stimuli-responsive elements, together with patient-specific molecular information, may improve precision nanomedicine.

13.6 Manufacturing, scalability and reproducibility

Clinical translation requires scalable production of nanoparticles with tightly controlled size, shape, ligand density and ligand organization [200]. Because small manufacturing variations can alter receptor clustering and pathway bias, standardized fabrication methods and rigorous quality control are essential for reproducible biological performance.

13.7 Regulatory and safety considerations

Successful clinical translation requires comprehensive evaluation of nanoparticle safety, efficacy and quality [201]. Potential toxicity may arise from nanoparticle composition, degradation products or membrane-disruptive delivery

strategies, while long-term biodistribution and tissue accumulation must also be assessed. Integrating regulatory and safety considerations early in nanoparticle design is therefore essential for successful development of pathway-selective nanomedicine.

14. FUTURE DIRECTIONS AND EMERGING FRONTIERS IN PATHWAY-SELECTIVE NANOMEDICINE

Pathway-selective nanomedicine is rapidly advancing through innovations in nanotechnology, molecular biology and computational science. While current approaches demonstrate that receptor nano-clustering can bias endocytic pathways, future strategies aim to achieve programmable, adaptive and clinically translatable control over receptor organization and intracellular trafficking [202].

14.1 Programmable control of receptor nano-clustering

Programmable nanostructures, particularly DNA origami, enable nanometre-scale control of ligand spacing, geometry and symmetry, allowing precise regulation of receptor clustering and endocytic pathway selection [203]. By matching ligand organization to receptor spatial preferences, these platforms optimize cooperative binding, reduce steric constraints and provide powerful tools for systematically engineering cellular uptake [204].

14.2 Synthetic receptor engineering and orthogonal targeting systems

Synthetic biology enables the design of receptors with programmable clustering behavior, membrane localization and intracellular trafficking motifs [205]. Orthogonal receptor-ligand systems further improve specificity by eliminating interference from endogenous signaling, allowing engineered cells to respond selectively to designed nanoparticles and enhancing therapeutic precision.

14.3 Multi-pathway and sequential targeting strategies

Future nanoparticles are likely to engage multiple endocytic pathways sequentially rather than relying on a single uptake mechanism [206]. Stimuli-responsive or dual-ligand systems can dynamically alter receptor interactions, enabling efficient cellular entry through one pathway followed by controlled intracellular trafficking or endosomal escape through another. These adaptive designs provide greater flexibility within complex biological environments.

14.4 Integration with immunotherapy and systems biology

Endocytic pathway selection strongly influences intracellular antigen trafficking, processing and presentation, making receptor nano-clustering highly relevant to cancer immunotherapy [207]. Whereas CME generally favors endolysosomal antigen processing, caveolar uptake or efficient endosomal escape enhances cytosolic antigen delivery, proteasomal processing and major histocompatibility complex class I (MHC-I) cross-presentation, thereby improving CD8⁺ T-cell activation and antitumor immunity [208]. These principles support rational design of cancer vaccines with enhanced antigen persistence, immune polarization, and memory T-cell formation. Integrating transcriptomics, proteomics, single-cell sequencing, and computational modeling further enables identification of regulatory networks controlling receptor clustering and pathway-selective endocytosis, facilitating intelligent nanomedicines capable of coordinated therapeutic delivery and immune modulation [209].

14.5 Advanced materials and responsive nanostructures

Stimuli-responsive nanomaterials that alter size, surface chemistry, or ligand presentation in response to pH, enzymes, temperature, or redox conditions offer dynamic control of receptor clustering [210]. Such systems remain relatively inert during circulation but activate within the tumor microenvironment, enhancing pathway-selective uptake while minimizing off-target interactions.

14.6 Precision nanomedicine and personalized therapeutic design

Patient-specific information on receptor expression, membrane composition, and tumor microenvironment increasingly enables personalized nanoparticle design [211]. Integrating artificial intelligence and machine learning with molecular profiling can predict optimal nanoparticle configurations tailored to individual receptor clustering and endocytic characteristics, supporting precision nanomedicine.

14.7 Challenges and opportunities for clinical translation

Translation of pathway-selective nanomedicine remains limited by challenges including scalable manufacturing, reproducible nanoscale ligand organization, and long-term safety assessment [212]. Continued advances in manufacturing, quality control, regulatory science, and interdisciplinary collaboration will be

essential to transform mechanistic insights into clinically applicable nanotherapeutics [213].

15. CONCLUSION

The ability to direct nanoparticle internalization through specific endocytic pathways represents a major advance in targeted anti-cancer nanomedicine. Central to this concept is the recognition that receptor nano-clustering primarily regulates recruitment of endocytic machinery. By controlling the size, spatial organization, density and temporal stability of receptor assemblies, nanoparticles can selectively bias uptake toward clathrin-mediated endocytosis (CME) or caveolin-mediated endocytosis (CvME). This framework integrates ligand-receptor interactions, membrane mechanics, lipid-domain organization and cytoskeletal regulation into a unified strategy for engineering intracellular trafficking. CME provides rapid and efficient cellular entry but is intrinsically linked to endolysosomal degradation, whereas CvME exploits cholesterol-rich membrane domains to preserve sensitive therapeutic payloads and improve access to cytosolic and organelle-specific targets. Rational pathway-selective nanomedicine therefore requires coordinated optimization of nanoparticle size, geometry, ligand valency, ligand organization, surface chemistry and binding kinetics in accordance with receptor characteristics and membrane biophysics. These design principles must also account for tumor microenvironmental influences, systemic biological interactions and translational constraints to ensure in vivo performance. Advances in super-resolution imaging, single-molecule tracking and complementary computational modeling have transformed understanding of receptor nano-clustering and endocytic pathway selection, enabling a shift from empirical nanoparticle optimization toward predictive, mechanism-based design. Emerging technologies-including programmable nanostructures, synthetic receptor engineering, and machine learning-guided nanoparticle design-are expected to provide increasingly precise control over cellular entry and intracellular trafficking, supporting adaptive and personalized nanomedicine strategies. Despite substantial progress, important challenges remain in scalability, reproducibility, and clinical translation. Addressing these limitations will require continued integration of materials science, molecular biology, computational modeling, and regulatory science. Exploiting receptor nano-clustering as a programmable determinant of endocytic pathway selection provides a unifying design principle that moves nanomedicine beyond passive targeting

toward predictive control of cellular entry and intracellular fate, offering significant potential to improve the specificity, efficacy, and clinical impact of next-generation anti-cancer therapeutics.

ABBREVIATIONS

BAR-Bin/Amphiphysin/Rvs
CME-clathrin-mediated endocytosis
CvME-caveolin-mediated endocytosis
DNA-deoxyribonucleic acid
EGFR-epidermal growth factor receptor
EPR-enhanced permeability and retention
FLIM-fluorescence lifetime imaging microscopy
FRET-förster resonance energy transfer
GPCR-guanine di-phosphate protein coupled receptor
GPI-glycosylphosphatidylinositol
HER-human epidermal growth receptor
MD-molecular dynamics
PEG-polyethylene glycol
RNA-ribonucleic acid
RTK-receptor tyrosine kinase
SEM-scanning electron microscopy
SPT-single-particle tracking
TEM-transmission electron microscopy
TfR-transferrin receptor

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Anamika Dutta contributed to writing original content. Trilochan Satapathy reviewed and edited the first draft of the manuscript. and editing. Poonam Sahu contributed to the visualization.

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