



Original Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Nanocarrier Targeting Is Governed by Biological Access Before Ligand Affinity

João Silva^{1*}, Pedro Costa¹, Ana Beatriz²

¹Department of Nanomedicine and Drug Delivery, Faculty of Pharmacy, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

²Department of Biological Barriers and Targeting, Faculty of Pharmaceutical Sciences, University of Campinas, Campinas, Brazil.

*Email: joao.silva@ufrj.br

ABSTRACT

Ligand–receptor affinity is often treated as the defining variable of active nanocarrier targeting, yet affinity can operate only after a carrier remains systemically available, reaches an accessible vascular segment, crosses or engages the endothelial barrier, traverses tissue, retains a biologically exposed ligand, and encounters a sufficiently available receptor. This Perspective argues that these preceding events should be treated as conditional access gates rather than background pharmacokinetics. Evidence from dose-dependent clearance, vessel-resolved permeability, transendothelial transport, tumour exit, interstitial pressure, macrophage redistribution, protein-corona remodeling, and single-particle ligand measurements shows that failure can arise before molecular recognition is possible. We therefore distinguish intrinsic affinity from biological access, functional ligand availability, receptor encounter, avidity, internalization, and payload availability. The resulting access-before-affinity interpretation is proposed as an evidence-bounded diagnostic logic, not as a universal quantitative law. It predicts that apparent targeting failure should first be localized to the earliest limiting biological compartment before affinity is optimized or blamed.

Keywords: Nanocarrier targeting, Biological access, Protein corona, Vascular transport, Receptor encounter, Ligand avidity

INTRODUCTION

Active targeting is frequently described through a ligand attached to a carrier and a receptor expressed by a desired cell. That description is chemically useful but biologically incomplete. Targeted delivery is conditioned by systemic disposition, tissue entry, local transport, cellular uptake, and intracellular fate, each of which can eliminate particles before receptor binding becomes possible [1]. The analytical error is therefore not studying affinity; it is treating affinity as though it acts on every administered carrier.

Cancer nanomedicine illustrates the problem sharply because formulation design and biological stratification influence translation beyond ligand choice [2]. Likewise, active targeting depends on the combined properties of vehicle, cargo, ligand, and indication rather than a receptor pair in isolation [3]. The relevant distinction is between molecular recognition capacity and the opportunity for that recognition to occur.

Tumour accumulation further resists a simple passive-versus-active dichotomy. Physicochemical properties, vascular features, microenvironmental transport, and ligand-mediated interactions can all contribute, while the relative importance of particular mechanisms remains context dependent [4]. This Perspective therefore asks a

narrower question: before interpreting affinity as the dominant determinant of targeting, has the carrier achieved biological access to the receptor-bearing compartment?

The targeting cascade before receptor binding

Systemically administered nanocarriers first face pharmacokinetic and biodistribution constraints that determine whether they can reach a biologically relevant compartment at all. Contemporary analyses of nanomedicine disposition emphasize circulation, tissue accessibility, and clearance as determinants of in-vivo performance [5]. For targeting interpretation, these processes should be treated as prerequisites rather than merely covariates.

The prerequisite is not necessarily linear. In mice, nanoparticle tumour delivery changed nonlinearly when particle burden altered Kupffer-cell uptake, demonstrating that systemic availability can itself depend on dose and clearance saturation [6]. The reported particle-number range is model specific and should not be converted into a human threshold. More importantly, experimentally reducing a major hepatic sink increased circulating availability without eliminating overall delivery inefficiency [7]. Improving one access gate can therefore expose a downstream limitation rather than solve targeting globally.

This motivates a proposed cascade logic: systemic survival, vascular opportunity, tissue entry, intratissue transport, biologically functional ligand presentation, and receptor encounter should be interrogated as distinct but interacting conditions. The proposal does not assume independence, fixed ordering in every administration route, or a multiplicative probability model.

Circulation and vascular access

Carrier design changes systemic access before ligand affinity is ever sampled by the target cell. In comparative gold nanostructures, geometry altered interactions with the mononuclear phagocyte system and subsequent tumour accumulation [8]. Such findings do not identify one universally optimal shape, but they show that carrier-scale physical properties alter the population of particles still available for later targeting.

Biofluid exposure then transforms the surface that biology actually sees. Protein coronas can suppress intentional targeting, redirect interactions, or sometimes be exploited for delivery [9]. Corona composition is dynamic and depends on the surrounding biological environment rather than being a stable extension of the synthetic formulation [10]. Accordingly, ligand density at manufacture and ligand identity during circulation are not interchangeable descriptors.

The biological milieu also introduces translational heterogeneity. Inter-individual and disease-related differences in protein-corona formation have been proposed as clinically relevant sources of variation [11], while more recent syntheses emphasize that corona composition can alter tissue interactions and organ-targeting behavior [12]. These observations support a conservative inference: a chemically identical nanocarrier may not present an identical biological interface across compartments or patients. They do not establish a clinically validated corona-based targeting classifier.

Extravasation and interstitial transport

Reaching the vascular bed of a target tissue is not equivalent to entering that tissue. Imaging and modelling studies of solid tumours have challenged the assumption that nanoparticle entry can be explained mainly by large static endothelial gaps; active transendothelial processes were prominent in the systems examined [13]. This does not invalidate paracellular leakage in every tumour. It instead makes the route of endothelial crossing an empirical question.

The vascular opportunity itself is highly local. Machine-learning-assisted analysis of tens of thousands of individual vessels across multiple tumour models revealed substantial vessel-to-vessel and tumour-to-tumour differences in nanoparticle permeability [14]. Whole-tumour averages can therefore hide spatially restricted access. This heterogeneity also helps explain why the enhanced permeability and retention effect is difficult to treat as a uniform clinical property; its magnitude and reproducibility vary across tumours and experimental settings [15].

Transcytosis provides an additional route by which nanocarriers may cross endothelium in selected systems [16]. The important point for targeting logic is not that one pathway is universally dominant, but that access can depend on endothelial biology before a carrier reaches the receptor-bearing parenchyma. Entry is also reversible. Nanoparticles can leave solid tumours through lymphatic or blood-associated routes, with exit behavior influenced by particle properties such as size [17]. Local exposure is therefore a balance among arrival, redistribution, residence, and loss.

After extravasation, tissue mechanics become a new constraint. Shape and curvature affected nanoparticle transport against elevated interstitial pressure in experimental and theoretical work, indicating that physical transport can restrict penetration independently of ligand chemistry [18]. Intravital imaging further showed that tumour-associated macrophages can engulf and redistribute nanoparticles after extravasation, producing movement that differs from simple free diffusion [19]. Such redistribution may increase spatial spread without proving productive delivery to the intended malignant cell.

Abnormal vasculature, dense extracellular matrix, and stromal organization can further restrict penetration [20]. These barriers create stage-dependent design trade-offs: particle characteristics favorable for circulation or accumulation may be unfavorable for deep tissue movement, motivating state-changing or disintegrable systems [21]. A pancreatic tumour study using an iRGD-containing nanomedicine illustrates the same distinction experimentally, because enhanced penetration into tumour parenchyma was separable from mere arrival at the tumour site [22]. Thus, tumour accumulation is too coarse an endpoint to establish receptor access.

Biological identity and immune clearance

Even after a carrier reaches the correct tissue, its engineered ligand may not remain biologically exposed. Experiments comparing directly conjugated ligands with ligands positioned after formation of an equilibrated protein corona showed that serum exposure could substantially reduce targeting function and that altered ligand presentation could restore binding in the studied systems [23]. The relevant variable is therefore functional ligand accessibility in the biological state, not simply the amount chemically attached during manufacture.

Corona-mediated effects can also create interactions not anticipated from the synthetic surface. Reviews of organ-targeting nanodrugs describe cases in which adsorbed proteins suppress intended recognition or participate in new tissue-selective interactions [24]. This makes biological identity a state variable rather than a passive coating. Consistently, deliberate albumin-oriented corona engineering altered circulation, cellular uptake, and tumour accumulation of a liposomal system in a mouse model [25]. These effects remain formulation and model specific. Biological identity also intersects with cellular uptake mechanisms. Different corona compositions caused the same nominal nanoparticle classes to use different internalization routes across in-vitro cell systems [26]. A receptor assignment inferred from a bare particle can therefore become misleading after biofluid exposure. The proposed interpretation is that immune clearance and corona remodeling do more than reduce dose: they determine which surviving carrier states remain eligible for later receptor encounter.

The biological-identity problem therefore requires a representation that keeps engineered surface state, acquired identity, systemic loss, and later encounter competence analytically separate. **Figure 1** consolidates those transitions while distinguishing reported nano-bio interactions from the proposed inference that they regulate eligibility for receptor encounter.

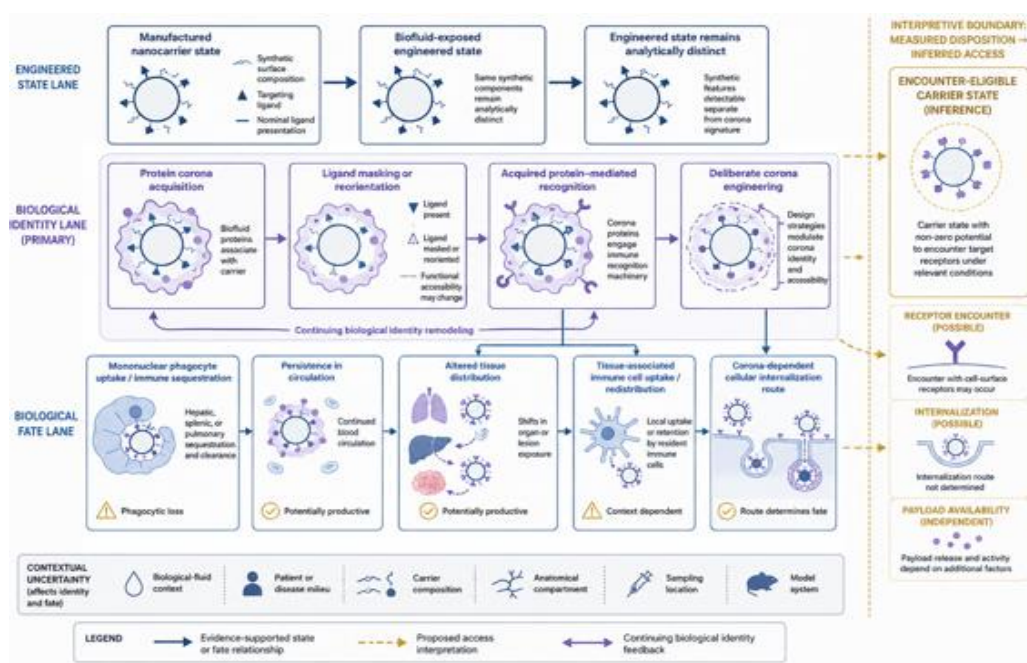


Figure 1. Carrier identity shifts that can alter eligibility for later receptor encounter

Receptor encounter as a spatial constraint

A receptor can influence targeting only when carrier and receptor become co-located at the appropriate spatial and temporal scale. Single-molecule imaging of cetuximab-functionalized nanoparticles showed that the number of functionally accessible antibodies differed from total conjugated antibodies and that targeting selectivity depended on both accessible ligand presentation and cellular receptor density [27]. Receptor abundance and carrier affinity are therefore insufficient descriptors when either partner is physically unavailable.

Spatial mismatch can arise at several scales. A carrier may remain intravascular while the receptor is extravascular; accumulate perivascularly while desired cells lie deeper in tissue; enter phagocytes rather than the intended cell; or reach the correct cell with its ligand masked by the acquired biological interface. Conversely, apparent low binding can reflect scarce local receptor exposure rather than weak intrinsic affinity. These cases can produce the same macroscopic observation—low target-cell uptake—while requiring different corrective experiments.

The proposed diagnostic principle is therefore to localize the failure before changing ligand chemistry. Measurements should distinguish circulating availability, vessel-level access, tissue depth, carrier-positive cell type, functional ligand exposure, and receptor availability. Because these look-alike failures cannot be adjudicated efficiently from a single uptake endpoint, **Table 1** organizes them as an exception-first encounter triage.

Table 1. Proposed encounter-failure triage for separating inaccessible target space from deficient molecular recognition

Misleading targeting pattern	Encounter-governing condition	Biological compartment and dominant sink	Observable transport or uptake signature	Strong competing diagnosis	Corrective test	Residual interpretation after mitigation
High blood persistence but little target-cell contact	Vascular opportunity remains limiting	Circulation and local endothelium	Persistent systemic carrier with sparse endothelial crossing	Receptor affinity is adequate but rarely sampled	Resolve vessel-level permeability and endothelial passage	Affinity cannot be adjudicated until vascular access is demonstrated
Strong tumour signal remains close to vessels	Spatial depth remains limiting	Perivascular interstitium, matrix, or pressure barrier	Tissue accumulation without distal parenchymal distribution	Apparent targeting reflects proximity rather than receptor selectivity	Measure distance-resolved carrier distribution and intended-cell occupancy	Perivascular arrival does not establish receptor encounter
Ligand-bearing carrier loses selective binding after biofluid exposure	Functional ligand exposure remains limiting	Acquired biological interface	Nominal conjugation persists while biologically accessible binding falls	Corona masking, steric restriction, or ligand reorientation	Quantify functional ligand exposure after biological equilibration	Weak targeting need not indicate weak intrinsic affinity
Receptor-rich tissue exhibits poor intended-cell uptake	Receptor co-localization remains limiting	Heterogeneous tissue and competing cell populations	Bulk receptor abundance without carrier–target-cell overlap	Accessible receptors lie outside the carrier's local distribution	Pair cell-resolved receptor mapping with carrier localization	Bulk expression cannot substitute for encounter evidence
Intracellular particle signal is concentrated in non-target phagocytes	Competing cellular sink dominates encounter	Tissue immune compartment	Extensive internalization in macrophages with limited intended-cell delivery	Uptake efficiency is being mistaken for target specificity	Identify carrier-positive cell populations and intracellular cargo location	Internalization alone does not establish productive targeting

Ligand density, avidity, and cellular internalization

Once encounter becomes plausible, molecular recognition variables regain importance—but as properties of a local biological interface rather than of the entire administered dose. Single-particle measurements of targeted

liposomes showed that available ligand density can differ from formulation input and that the relationship between ligand density and cellular interaction is system dependent [28]. Increasing nominal ligand loading should therefore not be assumed to produce monotonic gains in targeting.

Avidity further depends on geometry. Disk-shaped nanoparticles strengthened multivalent folate-mediated interactions in the studied system by increasing opportunities for simultaneous receptor engagement [29]. Nanoscale ligand organization can also alter receptor behavior: epidermal growth factor loading and nanoparticle clustering cooperatively influenced EGFR phosphorylation in cultured cells [30]. Such signalling results cannot be equated directly with therapeutic delivery, but they show that spatial organization matters beyond a single ligand–receptor affinity constant.

Cell-side context is equally important. In an engineered ErbB2 system, nanoparticle uptake varied jointly with ligand valency and receptor density [31]. The practical implication is that affinity, avidity, receptor abundance, and internalization should be measured at compatible biological and spatial scales. A carrier can display strong binding yet fail to make its payload available, or show high uptake predominantly in an unintended cell population. These distinctions make simple claims such as “more ligand improves targeting” analytically unsafe unless the measurement resolves biological identity, encounter, binding, internalization, and payload availability. **Table 2** converts such claims into testable adjudication problems so that upstream access failure is not misclassified as deficient affinity and downstream uptake is not misclassified as successful delivery.

Table 2. Proposed claim-to-test adjudication for ligand density, avidity, and cellular uptake

Targeting claim under review	Ligand-density or avidity classification basis	Dominant barrier or sink still plausible	Local biological identity and measurement scale	Minimum supporting observation	Strongest alternative explanation	Decisive test	Access-before-affinity endpoint allowed
More conjugated ligand improves targeting	Nominal surface loading	Ligand masking or steric crowding	Biofluid-equilibrated single-particle surface	Functionally accessible ligand increases rather than only formulation input	Added ligand is present but inaccessible	Quantify available ligand after biological exposure and relate it to cell interaction	Higher loading is meaningful only if functional presentation increases
High receptor expression predicts selective uptake	Receptor abundance plus encounter opportunity	Spatially inaccessible receptor or competing cells	Cell-resolved receptor and carrier localization	Carrier and receptor co-localize on the intended cells	Bulk expression hides inaccessible or off-target receptor pools	Joint receptor mapping and carrier-positive cell identification	Receptor abundance becomes interpretable only after co-location
Stronger multivalent binding proves better targeting	Increased avidity	Restricted transport, nonspecific retention, or altered membrane contact	Nanocarrier–cell membrane interface	Stronger binding occurs in the intended biological state	Geometry or clustering increases nonspecific attachment	Compare receptor-dependent and nonspecific binding under matched access conditions	Avidity may explain local retention but not upstream biological access
Greater internalization proves	Cellular uptake	Endosomal sequestration, wrong-cell uptake,	Intracellular and cell-type-resolved	Payload becomes available in the intended	Particle entry occurs without	Track carrier, payload, cell identity, and	Internalization remains an intermediate endpoint

productive delivery		or unavailable payload		cellular compartment	useful payload release	intracellular destination separately	until payload availability is shown
Geometry improves targeting because ligand affinity is higher	Multivalent contact opportunity	Altered margination, permeability, or uptake pathway	Carrier-scale geometry linked to receptor-scale contact	Geometry increases receptor-dependent engagement under matched exposure	Shape changes upstream transport or contact frequency instead	Separate transport exposure from receptor-binding measurements	Geometry may modify avidity without changing intrinsic ligand affinity

The access-before-affinity model

The evidence assembled across the preceding sections supports a change in how active nanocarrier targeting is interpreted, but not a new universal transport law. Circulation, vessel-level permeability, tissue penetration, biological identity, and functional ligand exposure can each determine whether affinity is ever sampled by the intended receptor [5, 14, 23]. We therefore propose the access-before-affinity model as a diagnostic ordering principle: before attributing poor targeting to weak ligand–receptor binding, establish that a biologically competent carrier reached the receptor-bearing microenvironment in sufficient spatial and temporal proximity for binding to occur.

The model separates four questions that are often conflated. First, did an administrable carrier state remain systemically available? Second, did that state enter and traverse the relevant tissue rather than terminate in a vascular, stromal, or phagocytic sink? Third, did the carrier retain functionally exposed ligand while encountering an accessible receptor? Fourth, after encounter, did multivalent binding, internalization, and payload handling support the intended pharmacological event? These are proposed interpretive gates, not independent probabilities. A carrier can revisit compartments, change identity, leave tissue, or be redistributed by cells, and several gates can be limiting simultaneously.

A further distinction concerns observability. Each gate requires measurement at its corresponding biological scale. Plasma exposure cannot substitute for endothelial passage, tissue fluorescence cannot identify which cells contain carrier, and total cell-associated signal cannot establish intracellular payload release. The proposed model therefore treats targeting claims as progressively conditional: evidence at an upstream scale permits interrogation of the next stage but does not certify it. This prevents a high-affinity formulation from receiving mechanistic credit for a delivery step that was never directly observed.

The model also permits bypasses. Local administration may diminish the importance of systemic clearance while leaving interstitial transport and cellular encounter unresolved; vascularly expressed targets may require little extravascular penetration; and immune-cell-directed carriers may deliberately exploit uptake that constitutes a competing sink during tumour-cell targeting. Biological access is therefore defined relative to the intended target compartment rather than being synonymous with tumour penetration.

This ordering changes failure attribution. Improving Kupffer-cell escape can reveal vascular or interstitial constraints; deeper tissue penetration can reveal receptor scarcity; and successful receptor binding can expose intracellular payload limitations. The proposed rule is therefore to identify the earliest demonstrable limiting gate, while allowing co-limitation when evidence cannot isolate one stage. Affinity remains mechanistically important within the subset of carriers that achieve encounter. “Access before affinity” consequently denotes conditional relevance, not biological superiority of transport over binding.

Because this argument is neither a simple linear cascade nor a catalogue of barriers, **Figure 2** separates measured carrier states, the proposed diagnostic gates, downstream recognition events, and the boundary beyond which stronger mechanistic or translational claims require additional validation.

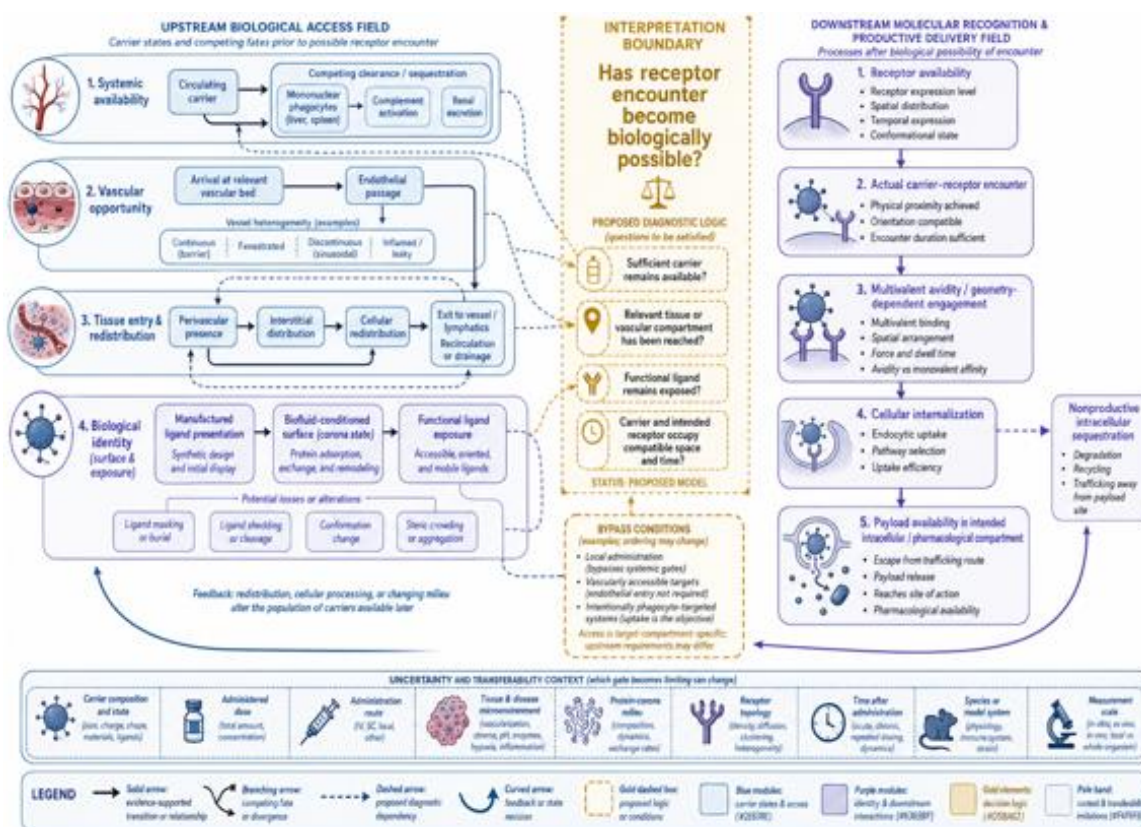


Figure 2. The decision boundary that determines when ligand affinity can explain nanocarrier targeting

Boundary conditions across tissues and diseases

The proposed ordering should not be mistaken for a fixed hierarchy shared by every tissue. Vessel permeability varies within and between tumours, interstitial pressure can oppose particle movement, and desmoplastic pancreatic models can impose severe distal-penetration constraints [14, 18, 22]. In some settings, the vascular gate may dominate; elsewhere, tissue mechanics, biological identity, receptor distribution, or intracellular handling may become limiting earlier. The order inferred from one biological context is therefore a hypothesis about that context rather than an intrinsic property of the nanocarrier.

The same caution applies across products and patients. Protein-corona composition can vary with biological milieu [11], and tissue-targeting interactions mediated by adsorbed proteins remain context dependent [12]. Route of administration, carrier composition, particle state, dose, disease stage, receptor topology, time after dosing, and measurement scale can consequently change which limitation becomes visible. A mechanism supported in one tumour, species, or nanoformulation should not be converted into a universal sequence.

An equally important boundary is conceptual: access-before-affinity does not predict that affinity optimization is futile. Once carriers are demonstrably co-localized with accessible receptors, functional ligand number and receptor density can materially influence selective interaction [27]. The model instead restricts the biological domain in which an affinity-based explanation is warranted. Its principal counterexample would be a system in which access is adequately demonstrated and matched, yet differences in receptor binding remain the dominant determinant of productive delivery.

Translational validation requirements

Translation requires more than demonstrating increased uptake in a convenient preclinical system. Analyses of nanotheranostic translation emphasize alignment among biological characterization, patient selection, manufacturing attributes, and clinical development [32]. For the proposed framework, this means measuring the compartment in which targeting is claimed. Plasma persistence, whole-organ accumulation, extravascular localization, intended-cell uptake, intracellular trafficking, and pharmacologically available payload should not be treated as interchangeable endpoints.

Recent mechanistic synthesis likewise indicates that solid-tumour delivery can involve multiple entry, retention, redistribution, and exit processes rather than one universal route [33]. Validation should therefore resolve

transport stages whenever the mechanistic claim depends on them. Whole-tumour accumulation may be useful pharmacokinetic evidence, but it cannot by itself establish receptor encounter or receptor-mediated delivery. Validation should also preserve formulation state over time. Measurements on the as-manufactured particle cannot automatically represent the carrier that reaches a vessel wall after biological exposure, while experiments in isolated cells cannot establish accessibility in intact tissue. An appropriately scaled validation package would align physicochemical characterization with biofluid-conditioned ligand accessibility, compartment-resolved biodistribution, cell-type localization, receptor availability, and payload fate at matched relevant times. The objective is not to require every assay for every product, but to ensure that the strength of a targeting claim does not exceed the biological level actually measured.

A practical translational test of the proposed model would compare matched formulations or biological contexts in which an upstream access variable and a receptor-interaction variable can be perturbed separately. Access-informed patient or model stratification is also a plausible research direction because biological stratification, vessel heterogeneity, and clinical-development constraints argue against assuming uniform exposure [2, 14, 32]. Such strategies remain prospective proposals; the approved evidence does not establish their ability to predict clinical benefit.

The strongest falsification test would be a setting in which carriers have demonstrably comparable systemic and tissue access, comparable functional ligand presentation, and comparable receptor co-localization, yet affinity optimization still explains the major difference in productive payload delivery. Conversely, if affinity changes remain ineffective until an upstream access constraint is corrected, that pattern would support the proposed ordering. Either result requires compartment-resolved measurements rather than retrospective inference from bulk accumulation.

CONCLUSION

Nanocarrier targeting should not be reduced to the chemistry of a ligand–receptor pair. Affinity becomes biologically meaningful only for carriers that survive sufficiently, reach the relevant vascular and tissue space, retain a functional targeting surface, and encounter an accessible receptor. The access-before-affinity model proposed here reorganizes those conditions into a diagnostic sequence for interpreting targeting failure without denying the downstream importance of avidity, internalization, or payload handling.

Its present value is methodological rather than predictive. It asks investigators to locate failure before redesigning affinity, distinguish accumulation from encounter, and separate receptor binding from productive delivery. Because barrier order can change across tissues, formulations, doses, disease states, and biological milieus, the framework should be tested rather than assumed. A more defensible targeted-nanomedicine claim is not merely that a ligand binds strongly, but that the carrier reached the biological situation in which that binding could matter.

ACKNOWLEDGMENTS: None

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

ETHICS STATEMENT: None

REFERENCES

1. Veiga N, Diesendruck Y, Peer D. Targeted nanomedicine: Lessons learned and future directions. *J Control Release*. 2023;355:446-57. doi:10.1016/j.jconrel.2023.02.010
2. van der Meel R, Sulheim E, Shi Y, Kiessling F, Mulder WJM, Lammers T. Smart cancer nanomedicine. *Nat Nanotechnol*. 2019;14(11):1007-17. doi:10.1038/s41565-019-0567-y
3. Gu W, Meng F, Haag R, Zhong Z. Actively targeted nanomedicines for precision cancer therapy: Concept, construction, challenges and clinical translation. *J Control Release*. 2021;329:676-95. doi:10.1016/j.jconrel.2020.10.003
4. Chen Z, Kankala RK, Long L, Xie S, Chen A, Zou L. Current understanding of passive and active targeting nanomedicines to enhance tumor accumulation. *Coord Chem Rev*. 2023;481:215051. doi:10.1016/j.ccr.2023.215051

5. Cabral H, Li J, Miyata K, Kataoka K. Controlling the biodistribution and clearance of nanomedicines. *Nat Rev Bioeng.* 2024;2(3):214-32. doi:10.1038/s44222-023-00138-1
6. Ouyang B, Poon W, Zhang YN, Lin ZP, Kingston BR, Tavares AJ, et al. The dose threshold for nanoparticle tumour delivery. *Nat Mater.* 2020;19(12):1362-71. doi:10.1038/s41563-020-0755-z
7. Tavares AJ, Poon W, Zhang YN, Dai Q, Besla R, Ding D, et al. Effect of removing Kupffer cells on nanoparticle tumor delivery. *Proc Natl Acad Sci U S A.* 2017;114(51). doi:10.1073/pnas.1713390114
8. Liu Y, Wang Z, Liu Y, Zhu G, Jacobson O, Fu X, et al. Suppressing nanoparticle-mononuclear phagocyte system interactions of two-dimensional gold nanorings for improved tumor accumulation and photothermal ablation of tumors. *ACS Nano.* 2017;11(10):10539-48. doi:10.1021/acsnano.7b05908
9. Kim W, Ly NK, He Y, Li Y, Yuan Z, Yeo Y. Protein corona: Friend or foe? Co-opting serum proteins for nanoparticle delivery. *Adv Drug Deliv Rev.* 2023;192:114635. doi:10.1016/j.addr.2022.114635
10. Wheeler KE, Chetwynd AJ, Fahy KM, Hong BS, Tochihuitl JA, Foster LA, et al. Environmental dimensions of the protein corona. *Nat Nanotechnol.* 2021;16(6):617-29. doi:10.1038/s41565-021-00924-1
11. Corbo C, Molinaro R, Tabatabaei M, Farokhzad OC, Mahmoudi M. Personalized protein corona on nanoparticles and its clinical implications. *Biomater Sci.* 2017;5(3):378-87. doi:10.1039/C6BM00921B
12. Chou WC, Lin Z. Impact of protein coronas on nanoparticle interactions with tissues and targeted delivery. *Curr Opin Biotechnol.* 2024;85:103046. doi:10.1016/j.copbio.2023.103046
13. Sindhvani S, Syed AM, Ngai J, Kingston BR, Maiorino L, Rothschild J, et al. The entry of nanoparticles into solid tumours. *Nat Mater.* 2020;19(5):566-75. doi:10.1038/s41563-019-0566-2
14. Zhu M, Zhuang J, Li Z, Liu Q, Zhao R, Gao Z, et al. Machine-learning-assisted single-vessel analysis of nanoparticle permeability in tumour vasculatures. *Nat Nanotechnol.* 2023;18(6):657-66. doi:10.1038/s41565-023-01323-4
15. Sun R, Xiang J, Zhou Q, Piao Y, Tang J, Shao S, et al. The tumor EPR effect for cancer drug delivery: Current status, limitations, and alternatives. *Adv Drug Deliv Rev.* 2022;191:114614. doi:10.1016/j.addr.2022.114614
16. Zhou Q, Li J, Xiang J, Shao S, Zhou Z, Tang J, et al. Transcytosis-enabled active extravasation of tumor nanomedicine. *Adv Drug Deliv Rev.* 2022;189:114480. doi:10.1016/j.addr.2022.114480
17. Nguyen LNM, Lin ZP, Sindhvani S, MacMillan P, Mladjenovic SM, Stordy B, et al. The exit of nanoparticles from solid tumours. *Nat Mater.* 2023;22(10):1261-72. doi:10.1038/s41563-023-01630-0
18. Jiang X, Xu S, Miao Y, Huang K, Wang B, Ding B, et al. Curvature-mediated rapid extravasation and penetration of nanoparticles against interstitial fluid pressure for improved drug delivery. *Proc Natl Acad Sci U S A.* 2024;121(22). doi:10.1073/pnas.2319880121
19. Lin ZP, Nguyen LNM, Ouyang B, MacMillan P, Ngai J, Kingston BR, et al. Macrophages actively transport nanoparticles in tumors after extravasation. *ACS Nano.* 2022;16(4):6080-92. doi:10.1021/acsnano.1c11578
20. Liu Y, Zhou J, Li Q, Li L, Jia Y, Geng F, et al. Tumor microenvironment remodeling-based penetration strategies to amplify nanodrug accessibility to tumor parenchyma. *Adv Drug Deliv Rev.* 2021;172:80-103. doi:10.1016/j.addr.2021.02.019
21. Zhu D, Yan H, Zhou Y, Nack LM, Liu J, Parak WJ. Design of disintegrable nanoassemblies to release multiple small-sized nanoparticles. *Adv Drug Deliv Rev.* 2023;197:114854. doi:10.1016/j.addr.2023.114854
22. Li X, Zhong H, Zheng S, Mu J, Yu N, Guo S. Tumor-penetrating iRGD facilitates penetration of poly(floxuridine-ketal)-based nanomedicine for enhanced pancreatic cancer therapy. *J Control Release.* 2024;369:444-57. doi:10.1016/j.jconrel.2024.04.004
23. Stordy B, Zhang Y, Sepahi Z, Khatami MH, Kim PM, Chan WCW. Conjugating ligands to an equilibrated nanoparticle protein corona enables cell targeting in serum. *Chem Mater.* 2022;34(15):6868-82. doi:10.1021/acs.chemmater.2c01168
24. Wang S, Zhang J, Zhou H, Lu YC, Jin X, Luo L, et al. The role of protein corona on nanodrugs for organ-targeting and its prospects of application. *J Control Release.* 2023;360:15-43. doi:10.1016/j.jconrel.2023.06.014
25. Li H, Yin D, Liao J, Wang Y, Gou R, Tang C, et al. Regulation of protein corona on liposomes using albumin-binding peptide for targeted tumor therapy. *J Control Release.* 2023;355:593-603. doi:10.1016/j.jconrel.2023.02.004
26. Francia V, Yang K, Deville S, Reker-Smit C, Nelissen I, Salvati A. Corona composition can affect the mechanisms cells use to internalize nanoparticles. *ACS Nano.* 2019;13(10):11107-21. doi:10.1021/acsnano.9b03824

27. Woythe L, Madhikar P, Feiner-Gracia N, Storm C, Albertazzi L. A single-molecule view at nanoparticle targeting selectivity: Correlating ligand functionality and cell receptor density. *ACS Nano*. 2022;16(3):3785-96. doi:10.1021/acsnano.1c08277
28. Chen C, Zhou Y, Chen C, Zhu S, Yan X. Quantification of available ligand density on the surface of targeted liposomal nanomedicines at the single-particle level. *ACS Nano*. 2022;16(4):6886-97. doi:10.1021/acsnano.2c02084
29. Dong N, Liu Z, He H, Lu Y, Qi J, Wu W. "Hook&Loop" multivalent interactions based on disk-shaped nanoparticles strengthen active targeting. *J Control Release*. 2023;354:279-93. doi:10.1016/j.jconrel.2023.01.022
30. Zhang Q, Reinhard BM. Ligand density and nanoparticle clustering cooperate in the multivalent amplification of epidermal growth factor receptor activation. *ACS Nano*. 2018;12(10):10473-85. doi:10.1021/acsnano.8b06141
31. Wang J, Min J, Eghtesadi SA, Kane RS, Chilkoti A. Quantitative study of the interaction of multivalent ligand-modified nanoparticles with breast cancer cells with tunable receptor density. *ACS Nano*. 2020;14(1):372-83. doi:10.1021/acsnano.9b05689
32. Gawne PJ, Ferreira M, Papaluca M, Grimm J, Decuzzi P. New opportunities and old challenges in the clinical translation of nanotheranostics. *Nat Rev Mater*. 2023;8(12):783-98. doi:10.1038/s41578-023-00581-x
33. Nguyen LNM, Ngo W, Lin ZP, Sindhwani S, MacMillan P, Mladjenovic SM, et al. The mechanisms of nanoparticle delivery to solid tumours. *Nat Rev Bioeng*. 2024;2(3):201-13.