



Original Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Targeted Nanomedicines Need Exposure Maps That Connect Organ Distribution to Cellular Entry

Luca Ferraro^{1*}, Matteo Ricci¹, Giulia Moretti², Paolo Greco³

¹Department of Targeted Nanomedicines and Biodistribution, Faculty of Pharmacy, University of Bologna, Bologna, Italy.

²Department of Organ Exposure and Cellular Entry, Faculty of Pharmacy, University of Turin, Turin, Italy.

³Department of Nanomedicine Design, Faculty of Pharmacy, Sapienza University of Rome, Rome, Italy.

*Email: luca.ferraro@unibo.it

ABSTRACT

Targeted nanomedicines are commonly evaluated through measurements that operate at different biological scales but are often interpreted as though they describe the same delivery event. Blood pharmacokinetics, organ biodistribution, local tissue accumulation, cellular association, internalization, intracellular trafficking, and payload availability answer different questions. This Perspective argues that the central translational problem is therefore not simply how much nanomaterial reaches an organ, but how exposure is partitioned across successive biological compartments and carrier states. Evidence from tumour nanomedicine, lipid nanoparticles, extracellular-vesicle systems, biomaterial transport studies, and intracellular imaging shows that major losses and redistributions can occur at vascular passage, tissue penetration, cell-type selection, uptake, and intracellular release. We propose that targeted-nanomedicine development should use multiscale exposure maps that retain these states separately and connect them only when carrier identity, biological compartment, measurement scale, and time are sufficiently aligned. Within this logic, molecular affinity becomes informative only after the carrier or its functional payload has obtained access to the relevant biological interface. The framework is not presented as a validated universal pharmacokinetic model. Rather, it is a measurement and interpretation architecture intended to expose where apparent delivery success reflects organ accumulation, where it reflects genuine cellular access, and where missing measurements prevent those states from being distinguished. Such maps could support more disciplined formulation comparison, mechanistic interpretation, and translational decision-making while preserving model-system and assay boundaries.

Keywords: Nanomedicine, Biodistribution, Cellular accessibility, Tissue microdistribution, Cell-specific uptake, Intracellular delivery

Received: 04 April 2025; **Revised:** 27 June 2025; **Accepted:** 29 June 2025

INTRODUCTION

Nanomedicine biodistribution is usually quantified through concentration or fraction-of-injected-dose measurements in blood, tumour, liver, spleen, lung, kidney, and other organs. These measurements are indispensable, but they describe only selected compartments of a delivery process. A large meta-analysis of nanoparticle distribution in tumour-bearing mice demonstrated both the value of quantitative organ pharmacokinetics and the substantial heterogeneity in tumour and major-organ accumulation across formulations and experimental conditions [1]. The pharmaceutical question that follows is more demanding: what fraction of the material counted in an organ is positioned so that it can participate in the intended pharmacological event?

Precision-nanoparticle design already recognizes that injected carriers encounter heterogeneous vascular, extracellular, cellular, and intracellular barriers [2]. Yet these barriers are frequently evaluated in separate experiments and reported through different denominators. A blood concentration may describe circulating carrier; organ radioactivity may represent intact particles, released label, degraded material, or mixtures depending on the system; tumour fluorescence may locate a carrier without identifying the receiving cell; and reporter expression may establish functional cargo delivery without directly reporting particle mass. The resulting measurements are biologically related but are not interchangeable.

This distinction has translational consequences. Industry analyses of anticancer nanomedicine development have emphasized adequate exposure at the relevant tissue and the importance of disease and patient context rather than formulation properties alone [3]. Likewise, the history of targeted cancer delivery shows that incorporating a high-affinity ligand into a carrier does not by itself establish efficient delivery to the intended cells in vivo [4]. Molecular recognition takes place downstream of multiple opportunities for clearance, sequestration, failed tissue entry, restricted diffusion, or uptake by competing cells.

We therefore propose an exposure-centred interpretation in which organ distribution is treated as one state within a multiscale chain rather than as the terminal delivery endpoint. The central question is not whether organ biodistribution should be replaced. It is whether it can be connected, with appropriate evidence boundaries, to tissue microdistribution, cellular accessibility, cell-type-specific uptake, and intracellular payload availability. The resulting exposure map is intended to preserve losses, redistribution, carrier-state changes, and measurement uncertainty instead of collapsing them into one nominal measure of targeting efficiency.

Why whole-organ biodistribution is insufficient

The distinction becomes concrete when organ- or tumour-level accumulation is compared with direct measurement at the intended cell. Dai and colleagues quantified ligand-coated nanoparticles in multiple mouse tumour models and found that only a very small fraction of the injected particles reached targeted cancer cells, while much of the material present in tumours remained extracellular or associated with other cellular compartments [5]. Their numerical efficiencies are specific to the tested nanoparticles, ligands, doses, and tumour models and should not be generalized to all targeted systems. The broader inference is nevertheless important: detection of a carrier within a tumour does not establish that the same carrier has accessed its intended cancer-cell surface.

The spatial organization of the tissue further separates these endpoints. Histopathological work has identified blood-vessel and tumour-associated-macrophage densities as predictive features of nanomedicine accumulation in studied tumour systems, while spatial modelling has shown that local tissue architecture contains information about where nanoparticles are found within heterogeneous tumour regions [6, 7]. These results do not establish a universal histological predictor of nanoparticle delivery. They instead show why a whole-tumour average can erase differences between locally accessible and poorly accessible regions.

This Perspective therefore distinguishes organ exposure from accessible exposure. Organ exposure answers how much measured material is associated with a macroscopic anatomical compartment. Accessible exposure asks where that material is located relative to the next biologically necessary interface. The latter may be vascular endothelium, extravascular stroma, a target-cell membrane, an endocytic compartment, or the cytosol, depending on the therapeutic mechanism. These states cannot be reconstructed retrospectively from a single whole-organ number unless validated relationships between the states are available.

The proposed multiscale exposure map is consequently not a replacement metric and does not impose a universal sequence on every nanomedicine. It is an accounting architecture. Its function is to state which exposure state has actually been measured, which transition is being inferred, and what evidence is missing before a downstream pharmacological interpretation can be made.

Blood and organ exposure

Blood pharmacokinetics and organ distribution remain the logical upstream layers because they define the amount of material available to reach downstream compartments. They must, however, preserve administration route and carrier state. Radiolabelled expansile nanoparticles administered intraperitoneally, for example, displayed a distribution pattern shaped by local administration and the associated tumour context [8]. Such findings are not evidence that intraperitoneal administration is generally preferable to intravenous administration. They demonstrate that route is part of the exposure condition and cannot be stripped from a biodistribution result when comparing formulations.

Organ kinetics can also be related to measurable physicochemical properties. A 2025 data-driven framework combining physiologically based pharmacokinetic modelling with quantitative structure–activity–style analysis linked nanoparticle descriptors including size, surface charge, and coating characteristics to selected organ kinetic parameters in healthy-mouse datasets [9]. Some predictions were strong within the analysed domain, whereas other concentration–time profiles remained difficult to reproduce. Computational exposure models should therefore be interpreted as domain-bounded bridges between formulation properties and measured pharmacokinetics, not as automatic predictors of downstream cellular delivery.

Formulation composition can itself redirect organ exposure. Selective organ targeting lipid nanoparticles demonstrated that adding defined SORT components could shift functional mRNA delivery and gene-editing activity among the liver, spleen, and lung in mice [10]. This establishes that organ tropism is not an immutable property of the lipid-nanoparticle class. At the same time, an organ-level expression signal represents a functional outcome and does not by itself specify how much intact carrier reached each constituent cell population or through which route.

Carrier surface architecture provides another example of coupled upstream effects. Bottlebrush-block-copolymer nanoparticles with altered nanoscale PEG surface topography showed changes in biological interactions, systemic circulation, tumour extravasation, distribution, and tumour-cell uptake relative to smoother comparator particles in the investigated platform [11]. The implication for exposure mapping is not that rough surfaces are universally superior. It is that a formulation variable may influence several linked exposure states simultaneously. Recording only the final tumour concentration can therefore conceal whether an observed difference originated in circulation persistence, vascular passage, interstitial transport, cellular access, or several of these processes together.

Tissue microdistribution

Once material reaches an organ or tumour vascular bed, microdistribution determines which local regions become reachable. In solid tumours, this transition cannot always be represented as passive leakage through static vascular gaps. Multimodal imaging across tumour models and human tumour material showed substantial nanoparticle transport through endothelial cells, while subsequent work identified a comparatively restricted subset of tumour endothelial cells associated with nanoparticle entry [12, 13]. These findings support an important scale distinction: vascular exposure is not equivalent to extravascular exposure, and the probability of crossing can vary spatially even within one tumour.

Local accumulation is also dynamic rather than purely retentive. Nanoparticles that enter solid tumours can subsequently exit through lymphatic routes and return toward the systemic circulation, with particle size influencing the observed behaviour [14]. An exposure map that records only entry and retained tumour burden therefore risks treating a transient state as a terminal one. Entry, retention, egress, and recirculation should remain conceptually separable until their relative contributions have been measured for the formulation and disease context under study.

Mechanistic resolution of endothelial passage adds another layer. Recent work examining pathways for nanoparticle transport across tumour endothelium found an important role for non-receptor macropinocytic transport in the studied systems, with pathway contributions dependent on particle and biological context [15]. This does not justify treating macropinocytosis as the universal route for nanoparticle extravasation. It illustrates why a map should distinguish a measured transition from an assumed mechanism and retain uncertainty when alternative pathways remain plausible.

Transport after extravasation is equally heterogeneous. Tumour-associated macrophages can migrate toward recently extravasated nanoparticles, engulf them, and redistribute them through tumour stroma; in the studied models, this active transport moved particles farther than passive diffusion alone, and the magnitude depended on particle size [16]. Macrophage uptake can therefore represent sequestration, redistribution, or potentially a therapeutically relevant cellular destination depending on the nanomedicine. Calling all macrophage-associated material a delivery failure would be as misleading as automatically treating it as successful targeting.

The extracellular matrix imposes an additional, tissue-dependent constraint. Measurements in tissue-specific extracellular matrices showed that matrix composition and concentration can strongly modify nanoparticle diffusion and subsequent cellular uptake and that PEG architecture can modify these effects [17]. The large fold changes reported in particular experimental settings should not be exported as universal *in vivo* improvements. The transferable point is that the amount of carrier physically present in a tissue and the amount capable of traversing its local matrix are distinct exposure quantities.

Cellular accessibility

Cellular accessibility begins where tissue localization stops being sufficient. We define it here as the condition in which a carrier, carrier-associated payload, or relevant released species can physically encounter the membrane environment of a cell capable of participating in the intended pharmacological mechanism. This is an explicitly proposed analytical state rather than an established universal nanoparticle metric. It is useful because material can be extravascular yet remain effectively unavailable to a target cell through distance, matrix confinement, sequestration, competing uptake, or an altered biological surface.

The carrier reaching this interface may also differ biologically from the engineered object that was injected. In mesenchymal-stem-cell-derived extracellular vesicles, protein-corona composition altered cellular uptake patterns and in vivo hepatic distribution, demonstrating that acquired surface identity can redirect biological interactions. Broader protein-corona scholarship likewise shows that adsorption of biomolecules can modify nanoparticle interactions with tissues and targeting systems [18, 19]. Extracellular-vesicle findings should not be numerically extrapolated to synthetic nanoparticles, but both bodies of evidence argue against assuming that the ligand display measured before dosing is the only surface state relevant at the cell-access stage.

Nor is corona formation necessarily a unidirectional loss of targeting function. In a mucosal system using transferrin-functionalized nanoparticles, interaction with mucus-derived proteins masked or displaced aspects of ligand-mediated targeting and reduced transport under some conditions, while manipulation of the resulting biological interface could alter transport behaviour [20]. The epithelial system differs fundamentally from tumour delivery, so its mechanism is not imported here as a tumour mechanism. It serves instead as a boundary example: the functional meaning of an engineered ligand depends on the biological compartment in which that ligand is being interpreted.

These observations motivate an access-before-affinity rule for targeted nanomedicines. The rule is proposed, not empirically validated as a universal law. It states that ligand–receptor affinity should be interpreted as a downstream determinant of delivery only after there is evidence that an appropriate carrier state reaches a receptor-accessible interface. High affinity cannot compensate for a carrier that remains in circulation, is sequestered in an unintended organ or cell, fails to cross the vascular barrier, becomes immobilized in matrix, or presents a biologically modified surface that no longer supports the intended interaction.

A static accessible/not-accessible label is still inadequate because access can evolve over time. **Table 1** therefore organizes cellular accessibility as a sequence of compartment-conditioned exposure states, each with a distinct measurement requirement and failure interpretation.

Table 1. Temporal exposure states that determine whether a targeted nanomedicine becomes physically accessible to its intended cellular interface

Circulation → relevant vascular bed Vascular lumen → extravascular tissue Extravascular space → cell-proximal microdomain			
Biological-compartment transition	Circulation → relevant vascular bed	Vascular lumen → extravascular tissue	Extravascular space → cell-proximal microdomain
Entry condition	Sufficient circulating carrier during a useful exposure window	Contact with a permissive vascular region	Successful passage beyond vessel-adjacent regions
Dominant barrier or competing sink	Clearance, off-target organ sequestration, carrier transformation	Endothelial restriction, heterogeneous entry sites, competing endothelial uptake	Dense matrix, stromal architecture, phagocytic sequestration
Transport or accessibility event	Vascular presentation of the carrier	Transendothelial passage	Diffusion or cell-mediated redistribution
Relevant carrier identity	Circulating intact carrier or defined circulating payload-associated state	Carrier state present at the endothelial surface	Carrier after local protein and cellular interactions
Measurement scale required	Blood plus organ-level kinetics	Vascular and microregional imaging	Tissue microdistribution

Persistence or delayed consequence	Short circulation can terminate downstream opportunity; prolonged circulation does not guarantee tissue entry	Entry may be spatially concentrated and reversible through later egress	Local trapping may persist; mobile cells may redistribute material
Article-level test	Is material available at the vascular interface when entry is possible?	Has organ exposure become true extravascular exposure?	Is the carrier positioned close enough to encounter the intended cell?

The distinction becomes visually clearer when access and molecular recognition are represented as separate decision dimensions rather than one targeting arrow.

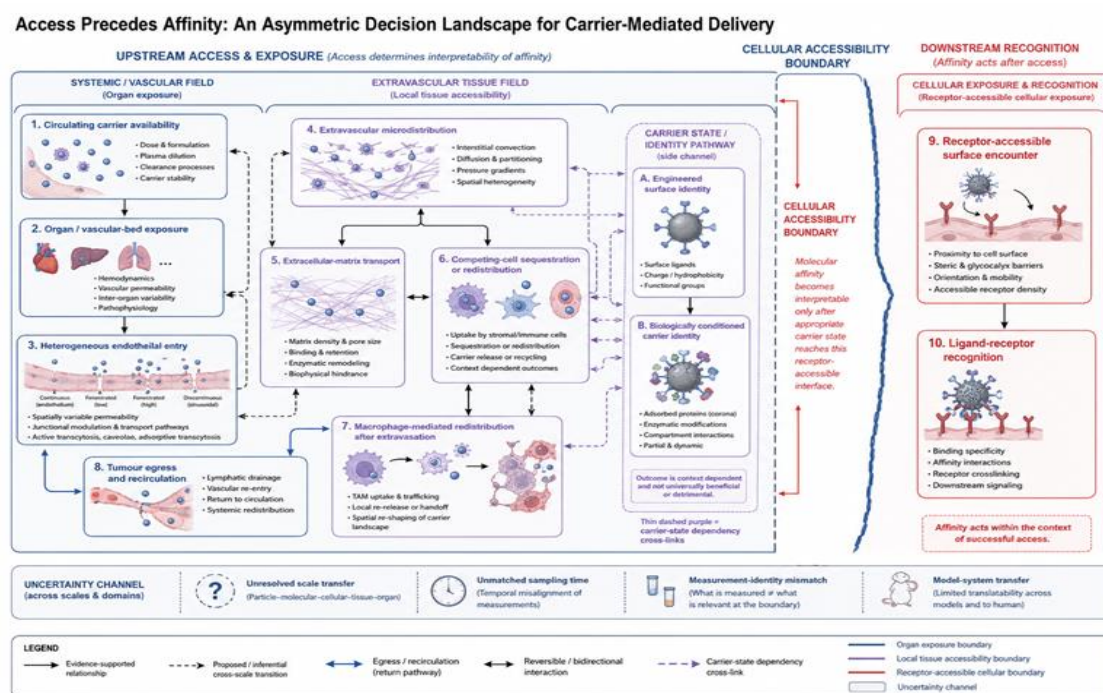


Figure 1. Cellular access determines when molecular affinity can become a meaningful delivery variable

Cell-type-specific uptake

Crossing the accessibility boundary still does not identify the cell that receives the carrier. Formulation chemistry can produce markedly different functional distributions among cell populations even when particles belong to the same general delivery class. A combinatorial library of ionizable lipid nanoparticles demonstrated that changes in lipid structure produced distinct cell-type mRNA-delivery profiles, including formulations with preferential expression in selected immune-cell populations [21]. These findings support cell-resolved formulation screening, but expression should not be interpreted as a direct measurement of intact-particle mass.

Cellular identity itself can alter uptake. In human macrophages derived from liver and blood, nanoparticle internalization varied substantially with macrophage phenotype, with regulatory and inflammatory polarization states displaying different uptake behaviour [22]. The reported subtype hierarchy is specific to the tested gold nanoparticles and experimental system. Its broader significance is that an organ label such as “liver” and even a cell-class label such as “macrophage” can remain too coarse when cellular state changes the probability of carrier capture.

Moreover, cell association is not equivalent to internalization. Single-particle microscopy has shown that many nanoparticles contacting a cell surface subsequently detach, whereas particles that do internalize can undergo rapid uptake followed by heterogeneous early intracellular trafficking [23]. Bulk fluorescence or flow-cytometric association can therefore combine biologically different events unless surface-bound and internalized fractions are discriminated.

For exposure mapping, cell-type-specific uptake should consequently be represented by at least three linked questions: which cell is physically accessible, which cell actually internalizes the carrier or cargo, and what material identity is being measured after uptake. Dose also matters because increasing total exposure may change

competition among cell populations or saturate some uptake processes, even when relative selectivity appears stable at another dose. No universal dose-response form is imposed here; the map instead requires dose to remain attached to the interpretation.

Table 2 shows those distinctions into delivery-design cases. Its framework endpoints are proposed and are intended to identify the experiment required to distinguish superficially similar uptake claims.

Table 2. Cell-resolved decision states for distinguishing carrier contact, internalization, and functional delivery

Delivery-design case	Evidence structure	Delivery-design case	Decision structure
Organ-selective functional signal	Governing biological condition Formulation chemistry redirects expression among organs or cell populations	Competing uptake among cell populations	Governing biological condition Different accessible cells have different internalization propensities
	Carrier state and compartment Administered carrier → organ-associated carrier/cargo		Carrier state and compartment Cell-proximal carrier → cell-associated carrier
	Dose dependence that must remain visible Expression pattern may change with formulation and dose		Dose dependence that must remain visible Higher exposure may amplify competing sinks as well as target-cell uptake
Phenotype-dependent uptake within one cell class	Discriminating experiment Pair organ-level functional readout with cell-resolved carrier or cargo measurement	Surface binding without productive entry	Discriminating experiment Quantify uptake in target and major competing cell types at matched dose and time
	Translation boundary Functional expression does not identify all cells containing intact carrier		Translation boundary Cell distributions are model- and formulation-dependent
	Proposed exposure-map endpoint Organ tropism established; cell-specific mass distribution unresolved		Proposed exposure-map endpoint Cellular partitioning measured
Productive cell entry	Governing biological condition Activation or differentiation state changes nanoparticle interaction		Governing biological condition Particle reaches the membrane but does not internalize
	Carrier state and compartment Cell-associated carrier at phenotypically distinct membranes		Carrier state and compartment Receptor-accessible or nonspecifically associated surface state
	Dose dependence that must remain visible Dose may obscure subtype differences if only pooled cells are analysed		Dose dependence that must remain visible Increasing dose can increase association without proportional entry
	Discriminating experiment Resolve phenotype markers and nanoparticle uptake in the same cells		Discriminating experiment Distinguish surface-bound from internalized particles with time-resolved methods
	Translation boundary Subtype hierarchy cannot be transferred automatically across particle classes		Translation boundary In vitro uptake kinetics do not define in vivo kinetics
	Proposed exposure-map endpoint State-specific cellular exposure measured		Proposed exposure-map endpoint Accessibility demonstrated; internalization unresolved
Productive cell entry	Governing biological condition Carrier crosses the plasma membrane		
	Carrier state and compartment Internalized carrier in early endocytic compartments		
	Dose dependence that must remain visible Exposure must be interpreted with internalized fraction and sampling time		
	Discriminating experiment Direct intracellular localization or orthogonal uptake assay		
	Translation boundary Internalization still does not establish cytosolic payload availability		
	Proposed exposure-map endpoint Cell-type-specific uptake established		

Note: The exposure-map endpoints are proposed synthesis. They are not a validated scoring scheme, and no row implies a universal temporal sequence for all nanomedicines.

Intracellular availability

Internalization is another boundary rather than the endpoint of delivery. Three-dimensional super-resolution microscopy has demonstrated that intracellular nanoparticle numbers and spatial distributions can be resolved within whole cells and that surface modification can alter cellular accumulation and localization [24]. Such measurements add a subcellular exposure layer that is invisible to organ biodistribution or conventional bulk uptake assays. Yet even accurate intracellular localization does not establish that a drug has reached the molecular compartment required for activity.

This distinction is particularly consequential for nucleic-acid delivery. Endosomal uptake can retain therapeutic cargo in vesicular compartments where it remains separated from cytosolic or nuclear targets. A pH-responsive endosomal-escape platform incorporating nucleic-acid cargo demonstrated that engineered escape can increase cytosolic delivery and functional activity in vitro [25]. The result establishes separability between uptake and productive intracellular release in that system; it does not establish a universally applicable escape technology or clinical advantage.

The wider lipid-nanoparticle literature similarly identifies endosomal escape as a major determinant of functional nucleic-acid delivery and links escape behaviour to the structure of cationic and ionizable lipids [26]. However, the relationship among lipid chemistry, endosomal membrane interaction, cargo release, cell state, dose, and toxicity remains formulation dependent. A single measurement of total intracellular nanoparticle abundance is therefore insufficient to determine the fraction of payload that becomes pharmacologically available.

For the proposed exposure-map architecture, intracellular availability should specify the measured entity and its compartment. An intact carrier in an endosome, released RNA in the cytosol, a nuclear oligonucleotide, and a translated protein are different exposure states even when all originate from the same administered formulation. The transition between them should be represented only when supported by an appropriate measurement. This prevents an apparently successful sequence of organ accumulation and cellular uptake from being interpreted as productive drug delivery when the decisive intracellular transition remains unmeasured.

Linking mass balance across scales

A multiscale exposure map becomes useful only if measurements made at different biological levels are not treated as interchangeable. This problem is particularly visible for mRNA lipid nanoparticles, for which particle localization, delivered transcript, and translated protein can each be measured but represent different material or functional states [27]. A strong organ-level particle signal can coexist with limited intracellular transcript availability, whereas protein expression may demonstrate productive delivery without revealing the complete distribution of the administered carrier. The identity of the measured entity must therefore remain attached to every exposure value.

Mass balance should also include movement out of compartments. The observation that nanoparticles can leave solid tumours through lymphatic routes and subsequently recirculate means that tumour accumulation is a dynamic balance of entry, redistribution, retention, degradation, and exit rather than an irreversible sink [14]. The proposed framework consequently avoids treating the amount measured in an organ at one time point as the conserved quantity from which downstream cellular exposure can simply be calculated.

Instead, each scale transition should be written as a conditional relationship. Blood exposure constrains the material available to a vascular bed. Vascular passage constrains extravascular exposure. Local transport constrains cell-surface accessibility. Internalization constrains intracellular carrier exposure, and intracellular trafficking constrains pharmacologically available payload. These statements define logical dependencies, not validated conversion factors. Where the fraction transferred between states has not been measured, the map should display an unresolved transition rather than supply an inferred efficiency.

Temporal alignment is equally important. An organ concentration measured hours after administration cannot automatically be combined with a cellular uptake measurement made at another time to reconstruct a continuous delivery trajectory. The same applies when radiolabel, fluorescence, RNA abundance, and protein expression are used as if they track the same physical entity. Cross-scale mass balance therefore requires compatible material identity, sampling time, biological compartment, and assay interpretation before two observations can be linked quantitatively.

Multiscale exposure maps

We propose a multiscale exposure map as a structured record of these distinctions. Its purpose is not to replace pharmacokinetic modelling or establish a universal sequence of nanoparticle transport. It is to make explicit what has been measured, which biological state the measurement represents, which transition is inferred, and where the chain of evidence becomes discontinuous.

The map begins with administered and circulating carrier states but retains formulation-dependent transformations rather than assuming that the injected material remains biologically unchanged. Organ exposure is then separated from vascular presentation, extravascular localization, cell-proximal accessibility, cell-specific association, internalization, intracellular trafficking, and payload availability. Evidence for endothelial heterogeneity, matrix-dependent diffusion, macrophage-mediated redistribution, corona-dependent changes in cellular interactions, and intracellular localization shows why these states cannot be collapsed into a single delivery-efficiency variable.

The architecture must also accommodate reverse and lateral movement. Nanoparticle egress can return material from tumour tissue toward systemic circulation [14]. Macrophages can redistribute particles after extravasation [16]. Protein adsorption can alter biological identity after administration [18]. These processes mean that a realistic exposure map is a state-transition system with feedback rather than a linear delivery pipeline.

Another requirement is explicit uncertainty. If a study reports organ fluorescence but does not distinguish intravascular from extravascular material, the vascular-to-tissue transition remains unresolved. If a cell-association assay does not discriminate surface-bound from internalized particles, internalization remains unresolved. If intracellular carrier localization is known but endosomal release is not measured, pharmacologically available payload remains unresolved. Missing states should remain visible because silently filling them creates mechanistic certainty that the experiment did not provide.

Table 3 therefore defines the design-space constraints required before adjacent exposure states can legitimately be connected.

Table 3. Design constraints for connecting nanomedicine exposure states without converting measurement gaps into inferred delivery

Exposure state being interpreted	Payload Measurement	Principal failure mode	Dose Article-level test
Scientific anchor	Payload Measurement	Principal failure mode	Dose Article-level test
Circulating material	Payload or carrier information required Identity of intact carrier, associated payload, or released label Measurement or design lever Orthogonal carrier and payload tracking	Measured signal no longer represents administered carrier	Dose and time condition Preserve sampling time and administered dose Article-level test Does the blood measurement identify the material assumed to reach the organ?
Organ-associated material	Payload or carrier information required Intravascular versus tissue-localized fraction where relevant Measurement or design lever Spatial or compartment-resolved imaging	Organ burden interpreted as tissue entry	Dose and time condition Match organ concentration to vascular exposure window Article-level test Has vascular presentation been distinguished from extravascular delivery?
Extravascular material	Payload or carrier information required Local position relative to vessels, matrix, and competing cells Measurement or design lever Microdistribution mapping and tissue phenotyping	Tissue average hides inaccessible microregions	Dose and time condition Preserve local exposure time Article-level test Is the measured material positioned where the intended cell can be reached?
Cell-associated material	Payload or carrier information required Target-cell identity and surface-bound versus internalized fraction Measurement or design lever Cell-resolved and internalization-specific assays	Association interpreted as productive uptake	Dose and time condition Report dose and uptake interval Article-level test Which cells actually internalize the carrier?

Intracellular carrier	Payload or carrier information required Subcellular compartment and carrier integrity	Endosomal accumulation interpreted as available drug	Dose and time condition Match intracellular state to sampling time
	Measurement or design lever Subcellular localization and trafficking measurements		Article-level test Has the carrier reached the compartment required for payload release?
Released or functional payload	Payload or carrier information required Molecular identity of released cargo and downstream readout	Functional signal treated as equivalent to particle mass	Dose and time condition Preserve delay between uptake, release, and response
	Measurement or design lever Cargo-specific and functional assays		Article-level test Is pharmacologically relevant payload availability directly demonstrated?

Note: The state-linkage tests are proposed interpretive rules. They are not experimentally validated conversion factors, pharmacokinetic parameters, or regulatory criteria.

These relationships require a figure that preserves carrier-state transitions, biological feedback, and unresolved boundaries rather than reducing delivery to a single directional arrow.

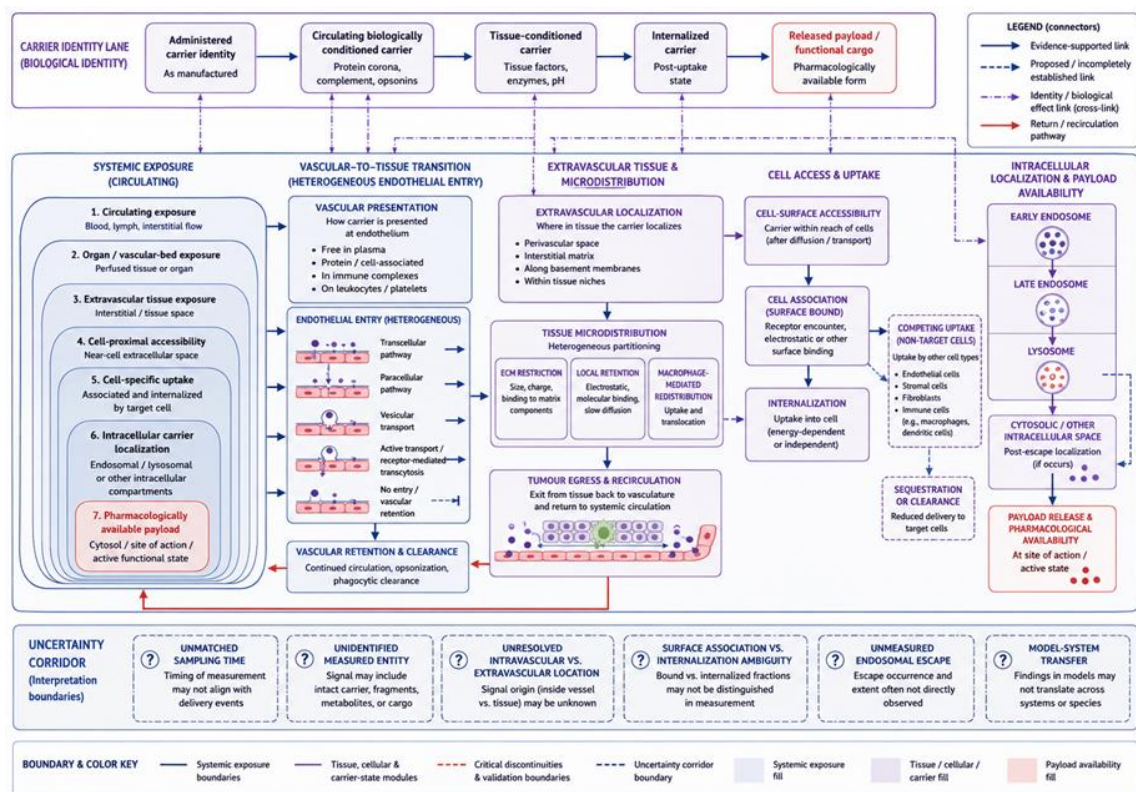


Figure 2. Exposure continuity fails where carrier identity, biological compartment, or measurement scale changes without validation

Measurement integration

Constructing such maps requires measurement integration rather than simply increasing the number of assays. Radiotracer biodistribution can provide sensitive whole-body and organ measurements, histopathology can resolve local tissue structure, single-particle imaging can distinguish association from internalization, and three-dimensional microscopy can locate nanoparticles within cells. These methods answer different exposure questions. Their outputs become mutually informative only when the biological object being measured remains identifiable across techniques.

Functional delivery assays create a particularly important interpretive boundary. Organ-level reporter expression after lipid-nanoparticle delivery can demonstrate that a cargo reached cells capable of producing the reporter, but it cannot reconstruct the complete particle distribution. Likewise, tracking particle, transcript, and protein levels in mRNA-LNP systems provides complementary rather than interchangeable information [27]. A useful

measurement program should therefore decide in advance whether the development question concerns carrier PK, tissue access, target-cell uptake, intracellular release, or functional payload activity and select measurements accordingly.

Computational models can assist by interpolating between measured states, identifying influential formulation properties, or testing consistency with physiological constraints. Their output should nevertheless remain visibly distinguished from direct measurement. The descriptor-based biodistribution model discussed earlier showed that several organ kinetic parameters could be predicted within its data domain while some concentration-time behaviour remained more difficult to reproduce [9]. A model should therefore not be allowed to convert an unmeasured cellular state into apparent mechanistic evidence merely because an organ-level prediction is accurate.

The practical objective is not complete measurement of every state in every experiment. It is strategic measurement of the transition most likely to determine the next development decision. If two formulations show similar tumour burden but different efficacy, cell-type partitioning or intracellular availability may deserve priority. If they differ strongly in blood persistence, detailed intracellular comparisons may be premature. Exposure maps can make that sequencing explicit.

Translational use

The translational value of this approach lies in identifying where a development claim exceeds the spatial or biological resolution of its evidence. Nanomedicine translation has long required alignment among formulation properties, relevant tissue exposure, disease biology, and patient selection [3]. A multiscale exposure map could make those dependencies more explicit by displaying which delivery state varies among models or patients and which remains unmeasured.

Tumour histopathology illustrates the possibility but also the boundary. Features including vascular and macrophage densities have been associated with or used to predict nanomedicine accumulation in particular experimental and human-tissue settings [6]. Such findings can motivate stratification hypotheses, but they do not establish that a histological feature predicts target-cell uptake, intracellular payload release, or therapeutic benefit for every carrier. Clinical use would require prospective validation at the specific exposure state relevant to the therapeutic mechanism.

The same restraint applies to cross-species modelling. Organ-level relationships learned in healthy mice cannot automatically be transferred to diseased human tissues, and cellular mechanisms identified in one tumour type may not dominate another. Exposure maps should therefore include model-system, formulation, disease-context, dose, and temporal boundaries as part of the interpretation rather than treating them as ancillary metadata.

Regulatory use is likewise prospective rather than established. The proposed architecture is not a qualification standard, validated biomarker framework, or accepted substitute for conventional pharmacokinetic, biodistribution, toxicological, or clinical measurements. Its nearer-term value is as an internal decision structure: it can show whether a claim of “targeted delivery” is supported by organ exposure alone, by direct cellular evidence, by intracellular payload evidence, or by a combination of measurements with explicitly validated links.

CONCLUSION

Targeted nanomedicine cannot be interpreted reliably through organ biodistribution alone because delivery success is partitioned across vascular, tissue, cellular, and intracellular states. Material detected in a target organ may remain intravascular, occupy inaccessible tissue regions, be captured by unintended cells, remain surface-associated without internalization, or enter cells without releasing pharmacologically available payload.

This Perspective proposes multiscale exposure maps to preserve those distinctions. The central principle is that each measurement should retain its material identity, biological compartment, time, and level of inference, and adjacent states should be connected only when the transition is measured or otherwise justified. Within this structure, molecular affinity becomes meaningful only after physical and biological access to the relevant interface has been established.

The framework is deliberately not a universal pharmacokinetic model or validated regulatory standard. Its purpose is to expose where evidence is continuous, where carrier state changes, where biological feedback alters distribution, and where missing measurements prevent stronger conclusions. Used in that bounded role, multiscale exposure maps could make formulation comparison and translational reasoning more precise by replacing the single question “Where did the nanoparticle go?” with the more pharmacologically relevant question “What

material reached which biological state, in which compartment, and was it still capable of producing the intended action?"

ACKNOWLEDGMENTS: None

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

ETHICS STATEMENT: None

REFERENCES

1. Chen Q, Yuan L, Chou WC, Cheng YH, He C, Monteiro-Riviere NA, et al. Meta-analysis of nanoparticle distribution in tumors and major organs in tumor-bearing mice. *ACS Nano*. 2023;17(20):19810-31. doi:10.1021/acsnano.3c04037
2. Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. *Nat Rev Drug Discov*. 2021;20(2):101-24. doi:10.1038/s41573-020-0090-8
3. Hare JI, Lammers T, Ashford MB, Puri S, Storm G, Barry ST. Challenges and strategies in anti-cancer nanomedicine development: An industry perspective. *Adv Drug Deliv Rev*. 2017;108:25-38. doi:10.1016/j.addr.2016.04.025
4. Rosenblum D, Joshi N, Tao W, Karp JM, Peer D. Progress and challenges towards targeted delivery of cancer therapeutics. *Nat Commun*. 2018;9(1):1410. doi:10.1038/s41467-018-03705-y
5. Dai Q, Wilhelm S, Ding D, Syed AM, Sindhvani S, Zhang Y, et al. Quantifying the ligand-coated nanoparticle delivery to cancer cells in solid tumors. *ACS Nano*. 2018;12(8):8423-35. doi:10.1021/acsnano.8b03900
6. May JN, Moss JI, Mueller F, Golombek SK, Biancacci I, Rizzo L, et al. Histopathological biomarkers for predicting the tumour accumulation of nanomedicines. *Nat Biomed Eng*. 2024;8(11):1366-78. doi:10.1038/s41551-024-01197-4
7. MacMillan P, Syed AM, Kingston BR, Ngai J, Sindhvani S, Lin ZP, et al. Toward predicting nanoparticle distribution in heterogeneous tumor tissues. *Nano Lett*. 2023;23(15):7197-205. doi:10.1021/acs.nanolett.3c02186
8. Colby AH, Kirsch J, Patwa AN, Liu R, Hollister B, McCulloch W, et al. Radiolabeled biodistribution of expansile nanoparticles: Intraperitoneal administration results in tumor specific accumulation. *ACS Nano*. 2023;17(3):2212-21. doi:10.1021/acsnano.2c08451
9. Wu J, Wick P, Nowack B. Data-driven prediction of nanoparticle biodistribution from physicochemical descriptors. *ACS Nano*. 2025;19(29):26425-37. doi:10.1021/acsnano.5c03040
10. Cheng Q, Wei T, Farbiak L, Johnson LT, Dilliard SA, Siegwart DJ. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing. *Nat Nanotechnol*. 2020;15(4):313-20. doi:10.1038/s41565-020-0669-6
11. Grundler J, Shin K, Suh HW, Whang CH, Fulgoni G, Pierce RW, et al. Nanoscale surface topography of polyethylene glycol-coated nanoparticles composed of bottlebrush block copolymers prolongs systemic circulation and enhances tumor uptake. *ACS Nano*. 2024;18(4):2815-27. doi:10.1021/acsnano.3c05921
12. 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
13. Kingston BR, Lin ZP, Ouyang B, MacMillan P, Ngai J, Syed AM, et al. Specific endothelial cells govern nanoparticle entry into solid tumors. *ACS Nano*. 2021;15(9):14080-94. doi:10.1021/acsnano.1c04510
14. 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
15. Wu JLY, Ji Q, Blackadar C, Nguyen LNM, Lin ZP, Sepahi Z, et al. The pathways for nanoparticle transport across tumour endothelium. *Nat Nanotechnol*. 2025;20(5):672-82. doi:10.1038/s41565-025-01877-5
16. 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

17. Cahn D, Stern A, Buckenmeyer M, Wolf M, Duncan GA. Extracellular matrix limits nanoparticle diffusion and cellular uptake in a tissue-specific manner. *ACS Nano*. 2024;18(46):32045-55. doi:10.1021/acsnano.4c10381
18. Liam-Or R, Faruqu FN, Walters A, Han S, Xu L, Wang JTW, et al. Cellular uptake and in vivo distribution of mesenchymal-stem-cell-derived extracellular vesicles are protein corona dependent. *Nat Nanotechnol*. 2024;19(6):846-55. doi:10.1038/s41565-023-01585-y
19. 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
20. Yang D, Feng Y, Yuan Y, Zhang L, Zhou Y, Midgley AC, et al. Protein coronas derived from mucus act as both spear and shield to regulate transferrin functionalized nanoparticle transcellular transport in enterocytes. *ACS Nano*. 2024;18(10):7455-72. doi:10.1021/acsnano.3c11315
21. Naidu GS, Yong SB, Ramishetti S, Rampado R, Sharma P, Ezra A, et al. A combinatorial library of lipid nanoparticles for cell type-specific mRNA delivery. *Adv Sci (Weinh)*. 2023;10(19). doi:10.1002/advs.202301929
22. MacParland SA, Tsoi KM, Ouyang B, Ma XZ, Manuel J, Fawaz A, et al. Phenotype determines nanoparticle uptake by human macrophages from liver and blood. *ACS Nano*. 2017;11(3):2428-43. doi:10.1021/acsnano.6b06245
23. Richards CJ, Burgers TCQ, Vlijm R, Roos WH, Åberg C. Rapid internalization of nanoparticles by human cells at the single particle level. *ACS Nano*. 2023;17(17):16517-29. doi:10.1021/acsnano.3c01124
24. Sheth V, Chen X, Mettenbrink EM, Yang W, Jones MA, M'Saad O, et al. Quantifying intracellular nanoparticle distributions with three-dimensional super-resolution microscopy. *ACS Nano*. 2023;17(9):8376-92. doi:10.1021/acsnano.2c12808
25. Narum S, Deal B, Ogasawara H, Mancuso JN, Zhang J, Salaita K. An endosomal escape Trojan horse platform to improve cytosolic delivery of nucleic acids. *ACS Nano*. 2024;18(8):6186-201. doi:10.1021/acsnano.3c09027
26. Mrksich K, Padilla MS, Mitchell MJ. Breaking the final barrier: Evolution of cationic and ionizable lipid structure in lipid nanoparticles to escape the endosome. *Adv Drug Deliv Rev*. 2024;214:115446. doi:10.1016/j.addr.2024.115446
27. Kang DD, Marks A, Morla-Folch J, Dong Y, Brown BD, Teunissen AJP. Targeting and tracking mRNA lipid nanoparticles at the particle, transcript and protein level. *Nat Biomed Eng*. 2025;9(10):1591-609. doi:10.1038/s41551-025-01511-8