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NANOPARTICLE DESIGN AT THE UNSTABLE BOUNDARY BETWEEN PHYSICOCHEMICAL CONTROL, BIOLOGICAL ADAPTATION, AND MANUFACTURABLE DRUG DELIVERY

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ABSTRACT

Nanoparticle drug-delivery systems are commonly designed through controllable material attributes, yet the identity governing their biological performance is altered as soon as they encounter complex physiological environments. This mismatch between engineered specification and acquired biological behavior complicates computational design, formulation optimization, safety assessment, and manufacturing translation. This article develops an original nanomedicine design-space theory for reasoning at that unstable boundary. The proposed Unstable Nano-Design Boundary treats a nanoparticle not as a fixed object but as a context-dependent sequence of states produced by interactions among engineered identity, biological context, acquired interfacial identity, immune and transport selection, and the manufacturability envelope. The approach integrates evidence on protein-corona formation, colloidal transformation, targeting disruption, process-dependent critical quality attributes, scale-up constraints, and multivariate formulation design. Its central contribution is a bounded design-space construct in which delivery, safety, manufacturability, and uncertainty must be evaluated jointly rather than collapsed into a single performance measure. The theory also distinguishes nominal formulation control from biological state control and separates computational prioritization from experimental confirmation and product reproducibility. The construct is not presented as an empirically validated model, universal taxonomy, regulatory criterion, or deployment-ready decision system. Its applicability remains conditional on material class, payload, route, disease state, host variability, exposure history, analytical method, and manufacturing platform. By making these dependencies explicit, the proposed framework may support more defensible model development, evidence mapping, experimental design, and translation planning for nanoparticle medicines.

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Introduction

Nanoparticle medicines are often discussed as engineered carriers whose composition, architecture, size, morphology, surface chemistry, payload, and release properties can be tuned toward a therapeutic objective. However, material novelty alone does not resolve the interacting biological barriers, disease heterogeneity, and translational constraints that determine whether a nominal design has pharmaceutical value; productive design therefore requires tighter coupling among materials science, biological measurement, delivery context, and product development [1-3]. The unresolved problem is not simply that many variables influence performance. It is that the variables controlled during formulation do not remain equivalent to the variables that govern behavior after administration.

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This distinction is especially important for computational and artificial-intelligence-based pharmaceutical science. A model may learn associations between nominal formulation descriptors and an experimental endpoint, yet those descriptors can become incomplete proxies when the particle enters a biological medium, acquires adsorbed biomolecules, changes colloidal state, or encounters route- and disease-specific barriers. Consequently, apparently "smart" behavior remains conditional on the biological environment and cannot be inferred from designed responsiveness or nominal surface features alone [4]. A high-performing predictor may therefore rank formulations within its data domain without explaining the mechanism, preserving the relevant state variables, or establishing reproducible usefulness across biological and manufacturing contexts.

The central evidentiary gap is the absence of a single design-space account that connects three normally separated forms of control. The first is physicochemical control: the ability to specify and measure the particle produced. The second is biological adaptation: the evolving interfacial and colloidal state generated by contact with proteins, lipids, cells, tissues, and clearance systems. The third is manufacturable delivery: the ability to reproduce a product whose critical attributes remain sufficiently stable for development, storage, scale-up, and use. Treating any one of these domains as the complete design problem encourages false optimization, because improvement in a local endpoint can be offset by altered immune recognition, transport, toxicity, process fragility, or batch variability.

This article therefore proposes the Unstable Nano-Design Boundary as an original theory-building construct. It is defined as the context-dependent region in which a reproducibly manufactured engineered identity is transformed by biological exposure, selected by immune and transport processes, and judged against simultaneous delivery, safety, manufacturability, and uncertainty objectives. The boundary is "unstable" because the relevant particle state can move with time, exposure history, route, host condition, process drift, storage, and measurement conditions. It is a boundary rather than a universal optimum because no isolated property, assay, model score, or delivery endpoint can establish acceptable overall design value. The construct organizes relationships and validation needs; it does not claim empirical validation or regulatory acceptance.

Why Nanoparticle Properties Change after Entering Biological Systems

A nanoparticle enters a biological system with an engineered identity, but it does not retain that identity as its only biologically relevant description. Contact with physiological fluids produces an acquired biological identity through dynamic adsorption and exchange at the surface, and that evolving interface can alter colloidal behavior, uptake, biodistribution, and targeting performance [5-7]. The transformation is not limited to a passive coating. It changes which chemical groups, ligands, charges, and nanoscale features are accessible to the surrounding biological environment, thereby modifying the effective object presented to cells and transport pathways.

The protein corona is a principal mechanism of this transformation because its composition and organization can govern cellular recognition and downstream biological outcomes [8]. Proteins with different affinities and exchange kinetics may enrich or deplete at the interface, while ligand masking, altered receptor accessibility, agglomeration, dissociation, payload leakage, or surface restructuring can further separate the acquired state from the nominal formulation. Thus, an analytically precise pre-administration characterization does not, by itself, identify the state that is recognized in blood, interstitial fluid, intracellular compartments, or disease-associated microenvironments.

The acquired state is also context-dependent rather than universally reproducible. Corona composition and consequence vary with particle material, surface chemistry, biological fluid, organismal condition, and measurement procedure [9]. Route of administration changes the first-contact medium; disease can change protein abundance and inflammatory state; prior exposure and dose sequence can alter the available biological environment; and sample handling can favor some interfacial states over others. These dependencies mean that "the corona" should not be treated as one stable formulation attribute. It is better represented as a family of time-indexed, context-indexed interfacial states with uncertain correspondence between experimental preparation and in vivo exposure.

Within the proposed theory, these changes are represented as a design delta between engineered identity and acquired biological identity. The delta includes changes in surface presentation, colloidal organization, accessibility of targeting motifs, degradation state, payload disposition, and the distribution of particle subpopulations. Its magnitude is not assumed to be directly measurable by one assay, nor is every transformation necessarily detrimental; some acquired states may improve circulation, transport, or tolerability. The scientific requirement is to identify which state variables change, under which contexts, with what persistence, and how those changes affect the decision objective. **Table 1** organizes the evidence, constructs, and interpretation boundaries needed to develop why nanoparticle properties change after entering biological systems within the article's central argument.

Table 1. Evidence domains, core questions, scientific requirements, and interpretation boundaries for why nanoparticle properties change after entering biological systems

Construct or evidence domain	Core question	Scientific basis	Role in the article	Failure or overclaiming risk	Boundary statement
Engineered identity	Which attributes are intentionally specified before biological exposure?	Composition, architecture, size distribution, morphology, surface chemistry, ligand presentation, payload	Establishes the controllable starting state against which biological	Treating a release specification or pre-administration measurement as a complete	Engineered identity is necessary for control but is not sufficient to predict biological behavior.

		state, release behavior, colloidal state, and process history define the nominal product.	transformation is interpreted.	description of in vivo identity.	
First-contact biological context	Which medium, route, dose history, and host state first interact with the nanoparticle?	Physiological fluids differ in protein and lipid composition, ionic strength, enzymatic activity, flow, cellular content, and disease-associated factors.	Defines the context in which the initial design delta begins to emerge.	Generalizing from one medium, species, route, or disease state to all intended uses.	Context must be specified; transfer across contexts requires separate evidence.
Acquired interfacial identity	Which molecules and structures become associated with or exposed at the particle interface?	Competitive adsorption, exchange kinetics, hard- and soft-corona organization, ligand masking, and surface restructuring alter biological presentation.	Connects material attributes to recognition, transport, uptake, and clearance.	Representing the corona as a fixed, uniform, or universally reproducible coating.	The acquired interface is dynamic, heterogeneous, and method-dependent.
Colloidal and structural transformation	Does the particle aggregate, dissolve, degrade, deform, restructure, or release payload during exposure?	Biological media can change colloidal stability, particle integrity, accessible surface area, payload retention, and subpopulation distribution.	Extends biological identity beyond protein adsorption to the full evolving particle state.	Inferring structural stability from a single time point or simplified medium.	Transformation must be evaluated over relevant times and exposure conditions.
Biological recognition and selection	Which evolving particle states are preferentially transported, internalized, sequestered, degraded, or cleared?	Cells and tissues respond to the acquired interface and transformed colloidal state rather than only to nominal descriptors.	Explains how small differences in acquired identity can generate divergent biological trajectories.	Equating association or uptake with mechanism, therapeutic delivery, or causal benefit.	Recognition, transport, and effect are distinct evidentiary claims.
Measurement correspondence	Do analytical measurements capture the state that exists at the relevant biological location and time?	Isolation, dilution, labeling, washing, and endpoint selection can perturb the interface or preferentially observe selected subpopulations.	Creates an explicit link between evidence quality and design-space inference.	Treating an assay output as a direct, unbiased representation of the in vivo state.	Measurement validity is conditional on sampling, perturbation, and biological relevance.
Design-delta uncertainty	How confidently can the difference between engineered and acquired identity be characterized?	Heterogeneity, temporal exchange, host variability, incomplete observability, and model assumptions limit state estimation.	Supplies the uncertainty term required for later multiobjective evaluation.	Converting uncertainty into an apparently precise composite score without calibration.	Uncertainty must remain visible and cannot be eliminated by nominal design control alone.

Physicochemical Design Variables and Manufacturing Constraints

Physicochemical design begins with variables that can be intentionally manipulated, but the feasible design region is bounded by how those variables are produced. Formulation composition, process parameters, and manufacturing platform jointly determine critical quality attributes and the practical feasibility of scale-up or scale-out [10-12]. Mixing sequence, flow regime, solvent exchange, energy input, temperature history, concentration, purification, and filling can influence particle size distribution, morphology, loading, residual components, surface presentation, colloidal stability, and release behavior. Accordingly, a computational design vector that omits process history may describe a desired particle while failing to describe a reproducible product.

Quality-by-design methods can organize the relationship among the quality target product profile, critical material attributes, critical process parameters, and critical quality attributes, thereby helping translate formulation knowledge into a controlled development strategy [13]. Their value within the proposed boundary is epistemic as well as operational: they force assumptions about controllable inputs, acceptable variability, and measurement strategy to become explicit. Nevertheless, quality by design does not remove biological uncertainty. A narrow and reproducible manufacturing range may still generate context-dependent coronas, transport patterns, immune interactions, or clearance behaviors that are not identifiable from release testing alone.

The design variables are also multivariate and entangled. Composition, component ratios, molecular weight, lipid or polymer chemistry, stabilizer concentration, aqueous and organic phase conditions, mixing intensity, purification, and storage can

interact rather than contribute independently. Design-of-experiments methods are therefore more suitable than one-factor-at-a-time adjustment for identifying interactions and defining a conditional robust region [14]. Even then, an experimentally efficient design region is not automatically a biological optimum: its boundaries depend on selected responses, model form, factor ranges, analytical precision, and whether the measured outputs represent the states that matter after administration.

The manufacturing constraint should therefore be treated as an active envelope around the nanoparticle design space, not as a downstream feasibility check. A candidate lies within that envelope only when its defining attributes can be produced, measured, stored, and reproduced with acceptable uncertainty under the intended platform. This framing changes computational optimization: infeasible compositions, fragile process combinations, poorly observable attributes, and designs whose biological function depends on uncontrolled batch variation should be penalized or excluded before claims of superiority are made. The resulting objective is not maximal nominal performance, but a controllable starting identity whose expected biological transformation and residual uncertainty can be evaluated within a reproducible product strategy.

Protein Corona Formation, Immune Recognition, Transport, and Clearance

Protein-corona formation connects the transformation of nanoparticle identity to biological recognition. Adsorbed biomolecules can expose, suppress, or reorganize molecular motifs that influence complement activation, opsonization, phagocytic engagement, receptor interactions, and the accessibility of targeting ligands. The immune system therefore encounters an acquired interface rather than an unmodified synthetic surface, and this acquired interface can change both immune recognition and targeting ability [15]. Within the proposed theory, immune interaction is not treated as a binary property such as “stealth” or “non-stealth.” It is a context-dependent selection process in which different particle states may be recognized, tolerated, internalized, sequestered, or removed at different rates.

Corona-dependent recognition affects more than immune-cell uptake. It can influence circulation, endothelial interactions, organ distribution, cellular internalization, intracellular processing, degradation, and eventual clearance [16]. These outcomes form a connected biological trajectory, but they should not be interpreted as interchangeable measures. Longer circulation does not alone establish effective tissue delivery; cellular association does not establish productive intracellular release; organ accumulation does not establish target-cell exposure; and reduced early clearance does not necessarily indicate improved safety. Each stage selects among particle subpopulations and can progressively separate the disposition of the administered dose from the behavior inferred from its average pre-administration properties.

The selection process is dynamic because the corona itself can be remodeled during circulation. Competitive adsorption and exchange can change the presentation of opsonins over time, meaning that circulation behavior may depend on sequential interfacial states rather than on the corona measured at one initial endpoint [17]. A particle characterized as weakly opsonized in one preparation may acquire a different recognition profile after dilution, transit through changing fluid environments, or interaction with cellular and extracellular surfaces. Static corona measurements remain informative, but they cannot be assumed to represent the complete exposure history. Temporal sampling, minimally perturbative analytical methods, and explicit documentation of the biological medium are therefore required when corona state is used as an explanatory or predictive variable.

Clearance must consequently be treated as a design outcome rather than as a residual event occurring after delivery. Renal filtration, hepatobiliary processing, mononuclear phagocyte-system sequestration, degradation, excretion, and long-term persistence depend on particle dimensions, composition, transformation, organ access, and biological processing [18]. Biodistribution and clearance are also coupled: strategies that alter circulation or tissue penetration may simultaneously change persistence, organ burden, degradability, and elimination pathways, with effects that vary across materials and administration routes [19]. The proposed construct therefore places immune recognition, transport, and clearance within one biological-selection layer while retaining distinct evidentiary requirements for each outcome. Optimization of one stage cannot establish acceptable performance across the complete disposition trajectory.

The Unstable Design Space Linking Material Control to Biological Adaptation

The unstable design space emerges because biological adaptation is continuous, partially observable, and history-dependent. In situ analysis indicates that the soft corona can evolve during exposure, making the biologically relevant interface dependent on time and prior interaction rather than solely on initial composition [20]. The state entering a capillary bed, tissue compartment, or intracellular environment may therefore differ from both the manufactured state and the state recovered through ex vivo isolation. In the proposed Unstable Nano-Design Boundary, time and exposure history are not secondary annotations; they are coordinates that determine which acquired identity is being evaluated.

Host biology supplies another moving coordinate. Interindividual and disease-associated differences in plasma composition can produce personalized coronas, creating the possibility that the same nominal nanoparticle formulation generates different acquired interfaces and biological behaviors across patients [21]. The proposed construct therefore contains seven linked components: engineered identity, biological context state, acquired biological identity, biological selection, manufacturability envelope, multiobjective decision surface, and uncertainty ledger. Engineered identity represents the controllable product state. Biological context conditions transformation. Acquired identity captures the evolving interface and colloidal state. Biological selection describes immune, transport, uptake, sequestration, and clearance processes. The manufacturability envelope constrains feasible starting states. The decision surface represents trade-offs among objectives, while the uncertainty ledger records what remains unobserved, variable, model-dependent, or unvalidated.

Instability also arises from entanglement among physicochemical variables. Size, charge, surface chemistry, morphology, ligand density, elasticity, colloidal organization, and process history may co-vary, so modifying one nominal attribute can change others and confound causal interpretation [22]. A model that assigns an outcome to one descriptor may therefore be learning a correlated formulation pattern rather than an isolated mechanism. This creates an identifiability problem for both experimentation and machine learning. Orthogonal characterization, deliberately varied formulations, causal hypotheses, sensitivity analysis, and reporting of correlated attributes are needed before a single variable is treated as the dominant driver of biological behavior.

Experimental findings illustrate how both host state and material mechanics can move the boundary. Altered cholesterol conditions can change corona composition and physiological response, demonstrating that a host metabolic factor may shift the biological identity of otherwise comparable particles [23]. Nanoparticle elasticity can also modify adsorption of apolipoprotein A-I and affect circulation lifetime, connecting a material property to corona formation and systemic fate [24]. These examples do not establish universal rules for cholesterol or elasticity. They show why the design space must represent conditional material–context–interface–fate relationships. The original contribution of the Unstable Nano-Design Boundary is to make that conditionality operational: a design claim is incomplete unless it identifies the engineered state, relevant biological context, expected transformation, selection outcome, manufacturing feasibility, uncertainty, and validation domain.

Multiobjective Evaluation of Delivery, Safety, and Manufacturability

Multiobjective evaluation begins with evidence traceability. Comparisons among nanoparticle designs require sufficiently complete reporting of material characterization, biological conditions, experimental procedures, analytical methods, and data interpretation to permit evaluation and replication [25]. Within the proposed theory, delivery, safety, manufacturability, and uncertainty are separate but interacting objectives. Delivery includes relevant exposure, transport, target-cell access, payload release, and pharmacological action. Safety includes hazard, exposure, persistence, off-target interaction, immune effects, and tolerability. Manufacturability includes process feasibility, control of critical attributes, stability, scale-up behavior, and batch reproducibility. Uncertainty includes measurement error, biological variability, model uncertainty, domain shift, and incomplete observation of acquired states.

Safety cannot be reduced to one viability assay, inflammatory marker, or short-term endpoint. Advanced assessment requires biologically relevant models, mechanistic measurements, exposure characterization, and integration of evidence across levels of organization [26]. A nanoparticle that improves one delivery endpoint may create a different risk through persistence, complement activation, tissue accumulation, degradation products, or altered intracellular processing. Conversely, a conservative design selected solely to minimize an isolated hazard signal may fail to produce meaningful therapeutic exposure. Safety evaluation must therefore remain coupled to intended route, dose pattern, target population, transformation state, and clearance pathway, while preserving the distinction between hazard identification and clinically relevant risk.

Multiobjective optimization offers a formal way to expose trade-offs that single-objective ranking conceals. Computational studies can identify non-dominated candidate regions in which improvement in one outcome requires compromise in another, but such regions remain conditional on model equations, selected objectives, parameterization, and input data [27]. The proposed decision surface should therefore be interpreted as a structured comparison of conditional alternatives, not as a universal score. A candidate may be preferable only within a stated route, biological context, manufacturing platform, and uncertainty tolerance. **Figure 1** presents the nanoparticle biointerface design delta, showing how the article's key components and boundaries are connected within multiobjective evaluation of delivery, safety, and manufacturability.

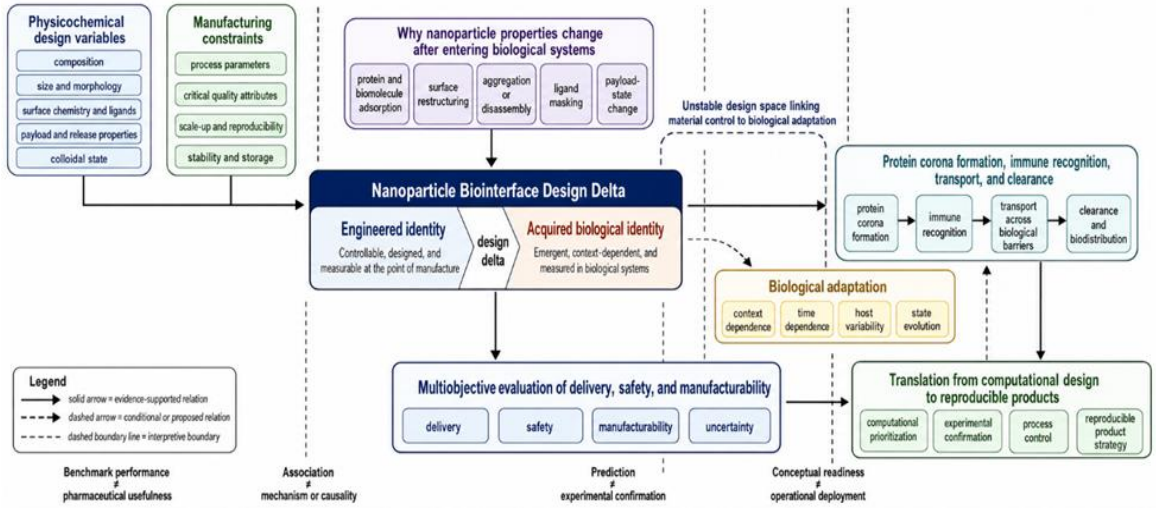

Figure 1. Nanoparticle Biointerface Design Delta

The figure is an original conceptual synthesis that organizes the article's central contribution across nanoparticle properties change after entering biological systems, physicochemical design variables and manufacturing constraints, protein corona

formation, immune recognition, transport, and clearance, protein corona formation, immune recognition, unstable design space linking material control to biological adaptation. Arrows and grouping indicate proposed or evidence-supported relationships rather than measured effect sizes. The figure does not represent a validated predictive model, regulatory determination, clinical recommendation, or deployment-ready system.

Machine-learning models may help prioritize formulations within this multiobjective space, but their prospective usefulness depends on the suitability of the training data, coverage of the intended domain, representation of uncertainty, and experimental confirmation [28]. Data-driven nanomedicine design can integrate heterogeneous descriptors and outcomes, yet retrospective prediction alone does not establish mechanism, generalization, or translational value [29]. Models should therefore report objective-specific performance rather than compress delivery, safety, and manufacturing attributes into an opaque score. Candidate rankings should be accompanied by applicability conditions, uncertainty estimates, sensitivity to missing variables, and a plan for prospective testing under the biological and process conditions relevant to intended use.

Translation from Computational Design to Reproducible Products

Computational design becomes translationally meaningful only when it is embedded in an iterative formulation and evidence-generation workflow. Machine learning can support property prediction, formulation screening, optimization, and prioritization, but its value depends on data curation, representation choices, validation strategy, and feedback from experiments [30]. The appropriate unit of learning is therefore not merely a list of nanoparticle compositions paired with endpoints. It should include process history, analytical methods, biological context, acquired-state measurements where feasible, and the uncertainty attached to both inputs and outcomes. Without these elements, a model may reproduce local dataset regularities while remaining insensitive to the variables that determine product reproducibility or biological adaptation.

Model-guided design loops can nevertheless generate experimentally testable candidates. Artificial-intelligence-guided optimization of lipid nanoparticles for pulmonary gene therapy demonstrates how formulation features and experimental outcomes can be connected through iterative candidate selection [31]. Such evidence supports the feasibility of bounded model-assisted design, but it does not establish transfer across organs, payloads, routes, disease states, lipid chemistries, or manufacturing conditions. The translation criterion is not whether a model identifies a promising candidate in one setting. It is whether the candidate and the decision logic remain interpretable, reproducible, and experimentally defensible within the intended product domain.

Computational screening, high-throughput experimentation, automated formulation, mechanistic modeling, and data-driven optimization may together improve the efficiency of nanomedicine discovery [32]. Their strongest role is to guide learning across design–build–test–learn cycles: proposing candidates, identifying informative experiments, updating uncertainty, and refining the feasible design region. Experimental work remains indispensable because biological identity, immune interaction, transport, payload action, process robustness, and stability cannot be confirmed through prediction alone. Each cycle should therefore test both expected performance and the assumptions that made the candidate appear favorable, including assumptions about context transfer, measurement correspondence, and manufacturing control.

Movement toward a reproducible product requires coordination beyond computational performance. Clinical translation depends on efficacy, safety, characterization, manufacturing, regulatory planning, and clinical positioning being developed as connected domains rather than sequential afterthoughts [33]. End-to-end translational frameworks further emphasize disease selection, product attributes, evidence generation, manufacturing, regulatory strategy, and commercialization as interdependent elements of readiness [34]. Within the proposed theory, these domains form the external boundary of the design space: a candidate remains conceptually promising until its engineered identity, biological transformation, selection behavior, manufacturing reproducibility, and use-context evidence are jointly tested. **Table 2** organizes the evidence, constructs, and interpretation boundaries needed to develop translation from computational design to reproducible products within the article's central argument.

Table 2. Evaluation, implementation, and research priorities arising from Nanoparticle Design at the Unstable Boundary between Physicochemical Control, Biological Adaptation, and Manufacturable Drug Delivery

Component or layer	Inputs	Core function	Expected output	Uncertainty or limitation	Validation requirement
Data and representation layer	Formulation composition, process history, critical quality attributes, analytical methods, biological context, exposure conditions, and outcome definitions	Create a traceable representation of the engineered and observed particle states	Curated, context-labeled development data suitable for analysis and modeling	Missing variables, inconsistent protocols, batch effects, measurement error, and selective reporting	Independent data review, protocol harmonization, provenance checks, and assessment of whether variables correspond to the intended decision
Computational prioritization layer	Curated data, mechanistic assumptions, candidate descriptors, optimization	Rank candidates or select informative experiments	Testable candidate set, predicted trade-offs, and explicit uncertainty	Domain shift, descriptor insufficiency, model misspecification,	Prospective evaluation against predefined objectives and comparison with appropriate non-

	objectives, and uncertainty estimates	within a bounded domain		correlated variables, and overfitting	model-guided selection procedures
Biological-transformation layer	Intended route, biological fluids, host or disease context, dose history, time, and engineered identity	Characterize transition from nominal formulation to acquired biological identity	Context- and time-specific description of corona, colloidal state, degradation, ligand accessibility, and payload disposition	Sampling perturbation, incomplete observability, interindividual variability, and uncertain in vitro–in vivo correspondence	Orthogonal measurements under biologically relevant conditions and confirmation that sampled states represent the intended exposure context
Biological-selection layer	Acquired identity, immune context, transport barriers, tissue environment, cell type, and clearance pathways	Determine how evolving particle states are recognized, transported, internalized, sequestered, degraded, or eliminated	Evidence-bounded disposition and target-access profile	Association may be mistaken for mechanism; uptake may not indicate productive delivery; organ accumulation may not indicate target-cell exposure	Mechanistic experiments, temporal disposition studies, relevant controls, and distinction among association, prediction, causality, and therapeutic effect
Manufacturing and control layer	Raw materials, unit operations, critical process parameters, scale strategy, storage conditions, and analytical controls	Produce a reproducible engineered identity within an explicit manufacturability envelope	Controlled product state, defined process range, stability strategy, and batch-comparability evidence	Scale-dependent mixing, raw-material variability, poorly observable attributes, storage drift, and process–attribute interactions	Repeated batches, scale-relevant process studies, stability testing, critical-attribute control, and predefined comparability criteria
Multiobjective decision layer	Delivery evidence, safety evidence, manufacturing evidence, uncertainty, intended use, and decision constraints	Compare non-dominated alternatives without collapsing distinct objectives into an unsupported composite score	Conditional candidate preference, trade-off map, or decision to obtain more evidence	Objective selection may be incomplete; weights may be subjective; model confidence may be uncalibrated	Sensitivity analysis, stakeholder review, prospective testing, and demonstration that conclusions remain stable under plausible assumptions
Translation and readiness layer	Disease rationale, product profile, experimental evidence, manufacturing capability, clinical positioning, and development constraints	Determine whether evidence supports progression to the next development stage	Bounded readiness statement and prioritized evidence gaps	Readiness is product- and context-specific; computational success can be mistaken for pharmaceutical usefulness	Stage-appropriate multidisciplinary review and independent confirmation of scientific, manufacturing, safety, and use-context assumptions
Learning and redesign layer	Experimental failures, batch variation, unexpected biological states, model error, and new contextual evidence	Update the design space, uncertainty ledger, and future experimental priorities	Revised hypotheses, narrowed or redirected design region, and documented reasons for change	Negative or contradictory findings may be excluded, producing biased learning	Versioned datasets, transparent decision records, replication where feasible, and explicit retention of failed or non-confirmatory results

Limitations and Boundary Conditions

The Unstable Nano-Design Boundary is a proposed conceptual construct, not a validated quantitative model. Its seven components organize relationships that are supported at different levels of evidence, but they are not claimed to be exhaustive, mutually independent, or universally measurable. Physicochemical attributes can be entangled, and observed associations may not identify the causal variable responsible for a biological effect [22]. The construct also does not specify universal weights, acceptance thresholds, or equations for balancing delivery, safety, manufacturability, and uncertainty. Such choices must be defined for a particular material class, route, payload, disease context, development stage, and decision purpose.

The supporting evidence is heterogeneous across particle systems, biological models, analytical techniques, and translational settings. Minimum-information reporting can improve transparency and reproducibility, but complete reporting does not itself establish biological relevance or product performance [25]. Likewise, a computational Pareto surface can reveal trade-offs

within a specified model without demonstrating that the modeled objectives capture the decisive in vivo or clinical variables [27]. The theory must therefore not be used to convert retrospective associations into mechanisms, model outputs into experimental confirmation, organ accumulation into therapeutic delivery, or formulation reproducibility into safety and efficacy.

Context dependence is an additional boundary rather than a removable nuisance. Patient-associated biological variation can alter acquired nanoparticle identity, but the predictive clinical value of personalized-corona measurements remains unestablished [21]. Translation challenges also differ among platforms, indications, routes, development organizations, and regulatory contexts [33]. The proposed construct cannot establish clinical benefit, regulatory acceptability, commercial feasibility, or operational readiness. It may support structured reasoning and study design only when users preserve uncertainty, document contextual assumptions, and require empirical validation before advancing consequential claims.

Research Agenda and Implementation Priorities

A first research priority is longitudinal characterization of the design delta. Studies should connect the manufactured state to time-resolved acquired identities and subsequent biological-selection outcomes under relevant routes, fluids, tissues, and host conditions. Because the soft corona evolves during exposure, measurements should examine state transitions rather than rely exclusively on isolated terminal samples [20]. Key questions include which transformed attributes persist, which are reversible, which are causally related to recognition or transport, and which analytical procedures perturb the state being measured. Comparative work should also determine when simplified media preserve the ordering of candidate formulations and when they produce misleading equivalence.

A second priority is the creation of interoperable evidence infrastructure. Experimental reports should document material identity, process history, biological context, analytical procedures, outcome definitions, excluded observations, and uncertainty at a level that supports replication and cross-study interpretation [25]. Computational workflows should preserve data provenance, separate training-domain performance from prospective usefulness, and connect prediction to experimental feedback [30]. Evaluation designs should include external or prospective testing where appropriate, analysis of domain shift, negative and failed formulations, and deliberate variation of correlated physicochemical attributes. Shared data structures should accommodate dynamic states rather than storing one static descriptor vector for each formulation.

Implementation should proceed through staged, reversible use. Early applications may focus on hypothesis generation, experimental prioritization, process-window exploration, and identification of evidence gaps rather than autonomous selection of development candidates. Integrated design–build–test–learn strategies can then update models and uncertainty as experimental evidence accumulates [32]. Progression toward product-development decisions should require multidisciplinary review spanning biological plausibility, pharmaceutical performance, manufacturability, safety, disease relevance, and translational strategy. End-to-end frameworks may help coordinate these domains, but each program still requires context-specific evidence and adaptation [34]. The implementation goal is therefore disciplined learning within explicit boundaries, not rapid deployment based on apparent predictive performance.

Conclusion

Nanoparticle drug delivery operates at an unstable boundary where engineered material control is continuously reshaped by biological adaptation and constrained by the requirements of reproducible manufacture. The proposed Unstable Nano-Design Boundary organizes this problem through engineered identity, biological context, acquired identity, biological selection, manufacturability, multiobjective decision-making, and an explicit uncertainty ledger. Its central implication is that no single measurement—whether particle size, corona composition, circulation, targeting, uptake, model accuracy, toxicity, or process yield—can establish overall design value. A defensible nanoparticle candidate must instead be evaluated as a context-dependent product whose transformation, biological trajectory, safety, reproducibility, and uncertainty are jointly examined. The construct is intended to guide theory building, experimental prioritization, computational representation, and translation planning; it does not constitute a validated predictive system, regulatory criterion, clinical recommendation, or universal design rule. Its value will depend on future empirical work that measures dynamic particle states, preserves context, tests causal assumptions, reports failure as well as success, and connects computational prioritization to reproducible pharmaceutical evidence.

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