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# Nanoparticle-Cell Interactions: Mechanisms of Uptake, Lysosomal Processing, and Implications for Nanomedicine Safety and Therapeutics

Komise pro obhajoby doktorských disertací v oboru molekulární biologie a genetika

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„The first principle is that you must not fool yourself – and you are the easiest person to fool. So you have to be very careful about that. After you've not fooled yourself, it's easy not to fool other scientists."

Richard Feynman

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# Summary

Nanoparticles are emerging as powerful tools in biomedicine, offering novel modalities for drug delivery, imaging, and disease modification. However, their clinical application remains limited, primarily due to an incomplete understanding of how nanoparticles interact with biological systems at the cellular and subcellular levels. This dissertation presents a comprehensive review of the cellular internalization of nanoparticles, their intracellular trafficking, lysosomal processing, and the resulting implications for therapeutic efficacy and safety.

The work integrates a critical review of the literature with extensive experimental datasets involving diverse nanoparticle platforms, including clinically approved superparamagnetic iron oxide nanoparticles, precision-synthesized polystyrene particles, and DNA nanostructures. These nanoparticles were characterized based on size, shape, surface chemistry, and functionalization. Both cellular and in vivo models were employed to investigate uptake kinetics, lysosomal trafficking, endosomal escape, and cytotoxic responses. Particular emphasis is placed on the lysosome as a key determinant of nanoparticle fate, serving both as a barrier to therapeutic delivery and as a potential target for treatment.

Key findings highlight the central role of the mononuclear phagocyte system in nanoparticle clearance, the formation of protein coronas that alter cellular uptake profiles, and the consistent convergence of nanoparticle processing within lysosomes. This convergence can impair autophagy and activate inflammatory signaling pathways. These insights are particularly relevant to nanomedicine applications in cancer therapy, lysosomal storage disorders, and central nervous system diseases.

The dissertation proposes a lysosome-centered model of nanoparticle–cell interaction, with the goal of engineering nanomedicines that exploit or bypass lysosomal pathways to enhance efficacy and reduce toxicity. The results contribute to the development of actionable design principles for nanoparticles and advance the broader understanding of nanomedicine safety, biodistribution, and translational potential.

# List of Abbreviations

3D Three-Dimensional
ADME Absorption, Distribution, Metabolism, and Excretion
ATR Attenuated Total Reflectance
CAM Cell Adhesion Molecule / Chorioallantoic Membrane (context-dependent)
CLIC/GEEC Clathrin-Independent Carriers/GPI-Enriched Early Endosomal Compartments
CME Clathrin-Mediated Endocytosis
CNS Central Nervous System
CTCF Corrected Total Cell Fluorescence
DLS Dynamic Light Scattering
DMEM Dulbecco's Modified Eagle Medium
DNs DNA Nanostructures
EPR Enhanced Permeability and Retention
FCS Fluorescence Correlation Spectroscopy
FDA Food and Drug Administration
FEME Fast Endophilin-Mediated Endocytosis
FOV Field of View
FTIR Fourier-Transform Infrared Spectroscopy
GFP Green Fluorescent Protein
I.V. Intravenous
LAMP1 Lysosomal Associated Membrane Protein 1
LIMP-2 Lysosomal Integral Membrane Protein Type 2
LNP Lipid Nanoparticle
MRI Magnetic Resonance Imaging
MSC Mesenchymal Stem Cell
MPS Mononuclear Phagocyte System
mTOR Mechanistic Target of Rapamycin
NIH National Institutes of Health
NPs Nanoparticles
PBS Phosphate-Buffered Saline
PI Propidium Iodide
PLL Poly-L-Lysine
PLGA Poly(lactic-co-glycolic acid)
RFP Red Fluorescent Protein
RPMI Roswell Park Memorial Institute Medium
SCI Spinal Cord Injury
SDS-PAGE Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SPIO Superparamagnetic Iron Oxide
SPION Superparamagnetic Iron Oxide Nanoparticle
TEM Transmission Electron Microscopy
TE Echo Time (in MRI)
TR Repetition Time (in MRI)
TAT Trans-Activator of Transcription (Peptide)
USPIO Ultrasmall Superparamagnetic Iron Oxide
UV–Vis Ultraviolet–Visible Spectroscopy
XTT 2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide

# 1. Introduction

## 1.1. Overview of Nanoparticle Applications in Biomedicine

Nanotechnology has reshaped biomedicine by enabling precision diagnostics and therapies [1-3]. Central to this shift are nanoparticles (NPs), e.g. materials typically 1–100 nm, whose high surface area, tunable chemistry, and multifunctionality differentiate them from bulk counterparts and make them powerful theranostic tools [1-3]. NPs can cross biological barriers, target specific tissues, and release payloads in controlled ways, improving efficacy and limiting toxicity, that are key goals of personalized medicine [1-3].

Two widely cited advantages are (i) improved delivery of poorly soluble active pharmaceutical ingredients (APIs) via lipid- or polymer-based nanocarriers, which can enhance bioavailability, reduce dose, cost, and toxicity [1, 4-7], and (ii) active or ligand-directed targeting of chosen cell types (e.g., immune or cancer cells) to raise specificity and reduce off target effects [1, 8, 9].

Clinical translation has advanced: by 2022 the U.S. FDA had approved 23 NP-based drugs, with many more in trials [9-11]. Most use liposomes, micelles, polymeric particles, or inorganic carriers [1-3]. Despite success, each platform has limitations.

***Definitions and regulatory context***

"*Nanoparticle*" is typically defined by size and physicochemical features, but upper size limits vary (100–1000 nm) [11-13]. The EMA demarcates 0.2–100 nm [14]. "*Nanomedicine*" refers to applying nanotechnology to diagnose, treat, or prevent disease, spanning delivery, imaging, diagnostics, tissue engineering, and regenerative medicine [13, 15, 16]. "*Nanodrug*" generally means a drug leveraging nanoscale materials (carriers or particles) to improve delivery, safety, or efficacy [11-13].

The FDA employs a flexible "*working definition*", focusing on (1) design features with at least one dimension ~1–100 nm and/or (2) size-dependent properties that may extend to ~1000 nm [17]. Emphasis is on engineered nanoscale structure and nanoscale-dependent function, enabling regulatory adaptability as technologies evolve. Key implications: products must be engineered, have at least one nanoscale dimension (or nanoscale internal features), and exhibit effects attributable to nanoscale properties, even if dimensions exceed 100 nm [17].

***Landscape of approved and emerging systems***

A systematic review identifies ~25 globally recognized approved nanodrugs, plus ~10 with national approvals (e.g., CosmoFer, Monofer in parts of the EU) [1, 6, 7, 10, 11, 18-22]. Major NP classes include lipid-based (liposomes, solid lipid NPs), polymeric (nanospheres, nanocapsules, dendrimers, micelles, polymersomes, nanogels), and inorganic (gold, silver, silica, iron oxide) (**Figure 1**) [1, 6, 7, 10, 11, 18-22].

Lipid systems (**Figure 1**) are biocompatible, encapsulate hydrophilic and hydrophobic cargos, and allow property tuning; they are widely used, including for nucleic acids (mRNA, siRNA, DNA) [1, 6, 7, 10, 11, 18-22]. Drawbacks include immunogenicity and potential nucleic acid degradation under certain conditions [23, 24].

Liposomes (described in the 1960s) encapsulate hydrophobic drugs in bilayers and hydrophilic agents in aqueous cores; PEGylation reduces opsonization and prolongs circulation, enabling delivery of chemotherapeutics, antibiotics, and gene therapy payloads [1, 6, 7, 10, 11, 18-22].

Lipid nanoparticles (LNPs), optimized for nucleic acids, typically contain an ionizable/cationic lipid, phospholipids, cholesterol, and PEG-lipids. They excel at transfection but can show limited loading versus liposomes and frequent liver/spleen accumulation [1, 6, 7, 10, 11, 18-22].
Polymeric NPs (**Figure 1**) support controlled release, co delivery, and versatile surface modification; drugs can be encapsulated, conjugated, or adsorbed [1, 6, 7, 10, 11, 18-22]. Preparation methods include emulsification/solvent evaporation, nanoprecipitation, ionic gelation, and microfluidics [25, 26]. Architectures (nanospheres, nanocapsules, dendrimers, micelles, polymersomes, nanogels) offer distinct profiles but face translational challenges in scalable manufacturing and reproducibility [27, 28].

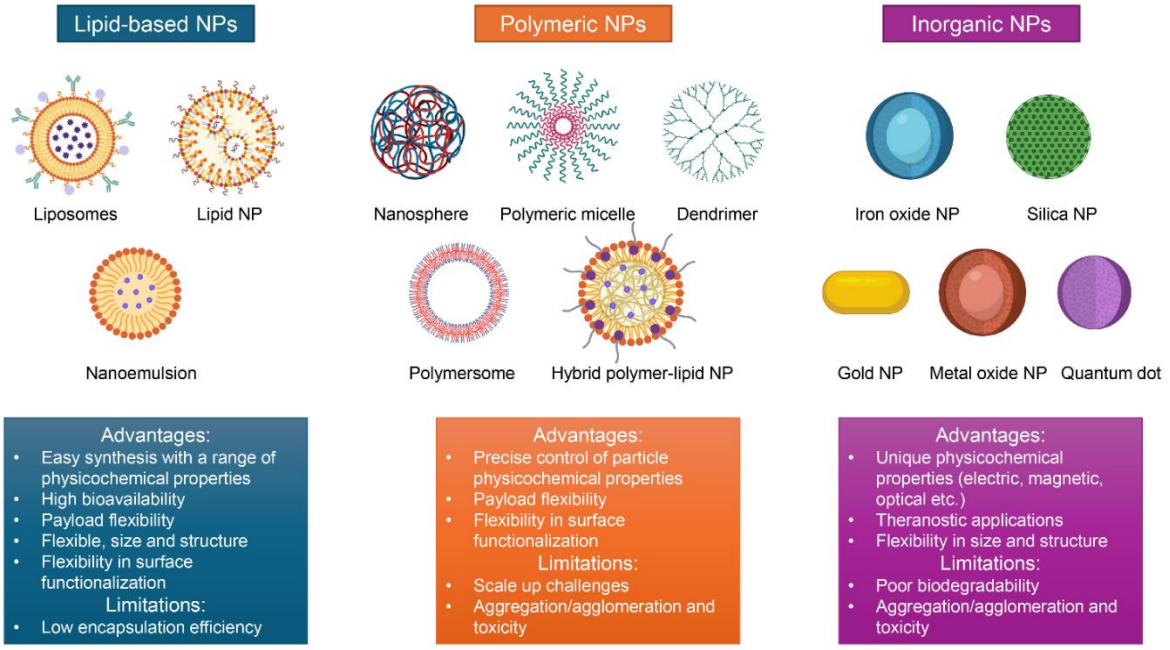


***Figure 1. Schematic illustration of major nanoparticle types used in clinically approved nanodrugs.*** *Each nanoparticle type includes several subtypes, a selection of which is highlighted here. These nanoparticle systems exhibit unique advantages and limitations related to cargo loading capacity, delivery efficiency, and patient-specific response profiles. Created with Bio-Render. Reproduced from ref [3]. CC BY 4.0.*

Inorganic NPs (metals, oxides, quantum dots, silica) offer unique optical/magnetic/electrical properties suited for imaging and stimuli-responsive applications (**Figure 1**) [29]. Iron oxide NPs are FDA approved mainly for iron supplementation; some MRI agents were withdrawn for safety/efficacy reasons [11, 20, 30]. Hafnium oxide NPs have EMA approval for use in squamous cell carcinoma [31, 32]. Gold NPs remain unapproved despite extensive research, with concerns about biodistribution, clearance, and genotoxicity, although trials continue [33, 34].
Iron oxide NPs (magnetite/maghemite) are clinically relevant due to biocompatibility and superparamagnetism, with applications in MRI, targeted delivery, hyperthermia, and antimicrobial strategies under investigation [1, 6, 7, 10, 11, 18-22].

### 1.2. Routes of Administration and Pharmacological Profiles of Nanoparticles

Nanomedicine pharmacology embodies NP-biological interactions by ADME (absorption, distribution, metabolism, excretion). Key determinants are mononuclear phagocyte system (MPS) clearance, stability, formation of protein corona,

size/charge, mechanism of uptake, and EPR effect [35]. ADME is NP class-dependent and not completely characterized [35].

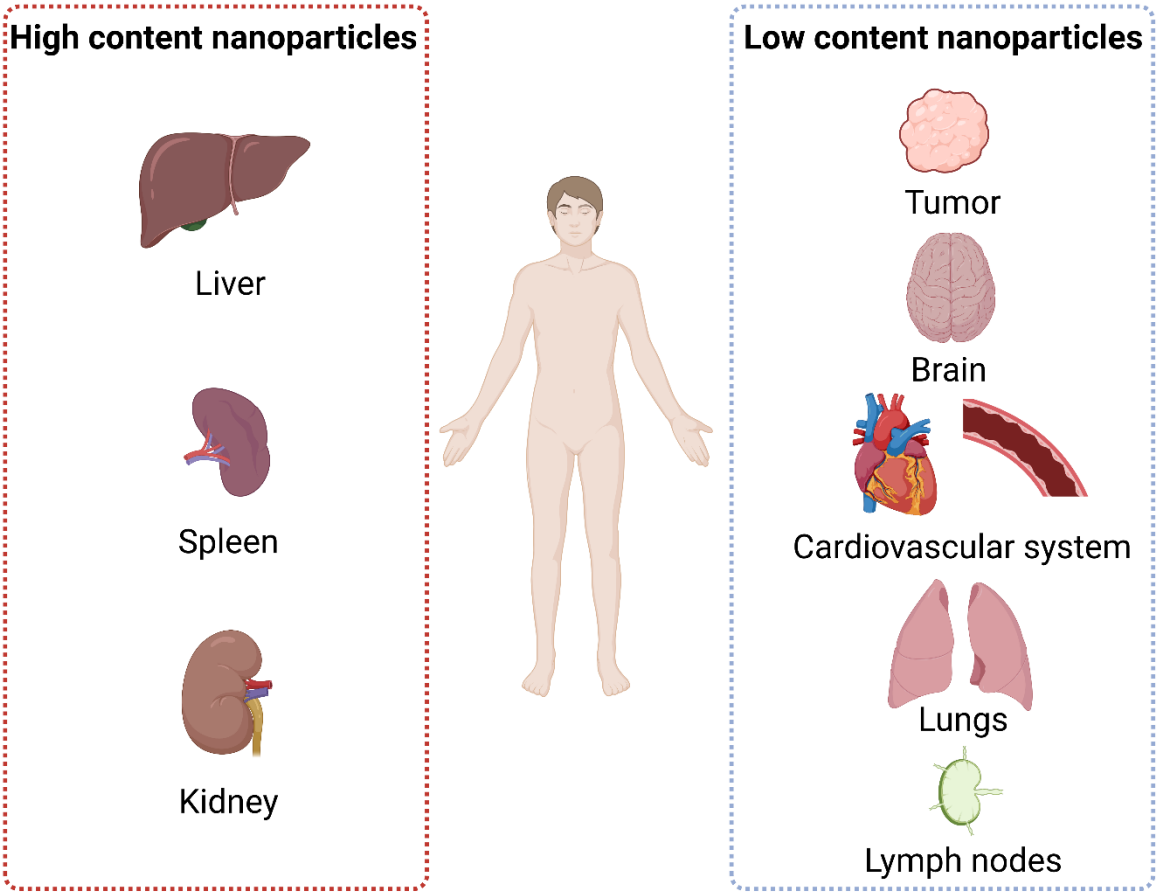


***Figure 2. Major sites of nanomedicines distribution in the body.*** *The high concentration of nanomedicines typically accumulates in the liver, spleen, and kidneys. Lower concentrations of nanomedicines are usually observed in the tumors, brain, cardiovascular system, lungs, and lymph nodes. Created with BioRender.com. Reproduced from ref [11]. Copyright 2023 Elsevier.*

Administration routes are oral, transdermal, ocular, nasal, pulmonary, and most notably intravenous (IV) injection [35]. Oral delivery must cross intestinal mucus and epithelium; surface engineering increases mucus penetration and transcytosis or paracellular movement through transient disruption of tight junctions [35, 36]. Transdermal methods avoid GI/first-pass metabolism; lipid NPs are skin-permeant and release-modulated [35, 37]. Ocular administration must overcome tight junctions and rapid clearance; formulations attempt to bind mucosa and resist enzymes, with topical/intraocular treatments explored [38-40]. Intranasal delivery is able to reach the CNS by epithelial endocytosis [41, 42]. Pulmonary delivery is aerodynamically size-dependent (1-5 μm optimum for bronchiolar deposition); inhaled NPs may be released into the bloodstream or trapped by alveolar macrophages [35, 43].

Clinically, oncology accounts for most nanomedicine indications, and IV administration is the most common (except for intramuscular mRNA LNP COVID-19 vaccines) [1, 44-49]. Formulation typically addresses three themes: leveraging EPR, extending circulation (preventing quick liver/spleen clearance), and achieving maximum antitumor effect with minimal systemic toxicity [50].

NPs rapidly adsorb biomolecules in blood to form a corona that controls aggregation, solubility, surface reactivity, and even protein conformation/activity [20, 51-56].

Corona formation can inhibit targeting ligands and alter enzyme activity [57, 58]. Pre-formed coronas or utilizing corona-mediated targeting (e.g., Onpattro hepatocyte uptake) are strategies to overcome unwanted effects of protein corona [20, 51-53]. "Selective organ targeting" (SORT) motifs are in the development to manage LNP biodistribution [59, 60].

EPR effect is found to be robust in rodent xenografts, augmented NP deposition and improved tumor delivery versus free drugs [49, 61-67]. So far, human tumor data are heterogeneous. Tumor ECM density and model variability make clinical relevance tricky; endothelial transcytosis may have dominance in entry in some cases [62, 68-74]. EPR-like phenomena under inflammatory conditions may be targetable (e.g., dexamethasone NPs in models of colitis) [75-77].

Beyond vasculature, NPs must penetrate ECM to reach cells; some exit via lymphatics to nodes, of possible use for vaccines and nodal metastases, though liver/spleen sequestration limits nodal dosing; size/charge engineering can help [78-82]. In general, IV NPs are predominantly accumulated in liver and spleen with secondary distribution to kidneys, heart, tumors, lungs, and other organs [1, 20, 44, 48, 83, 84].

In summary, the available literature indicates that most nanomedicines are administered intravenously, a route that typically leads to predominant accumulation in the liver and spleen, with secondary distribution to the kidneys, heart, tumors, lungs, and other organs (**Figure 2**) [1, 20, 44, 48, 83, 84].

This characteristic biodistribution, in turn, shapes which cell populations are most likely to encounter and interact with nanoparticles.

### 1.3. Hepatic Uptake and Excretion Pathways of Nanoparticles

NP pharmacokinetics and pharmacodynamics (PK-PD) is strongly controlled by clearance via the MPS, kidneys, and liver [1, 20, 44, 48, 83]. Even EPR based or actively targeted platforms show significant liver/spleen/kidney uptake, which is responsible for limited delivery to the tumor (~<1-2% ID) [83, 85].

Elimination pathways. IV NPs are eliminated through renal excretion (urine) or hepatobiliary pathways (bile-stool) [85-88]. Ultrasmall NPs (<6-8 nm hydrodynamic diameter) are preferentially filtered out by renal filtration; NPs greater than this used clinically are sequestered in the liver/spleen, then cleared via bile [1, 44-49].

Shape and surface control fate after phagocytosis. Inorganic NPs live longer than organic; small difference in size, shape, and chemistry markedly affect distribution and half-life [1, 20, 44, 48, 83, 89]. Reported elimination half-lives: Au, Ag, quantum dots, CNTs ~4-7 h; iron oxide up to 24 h or days; coatings alter distribution half-lives to minutes to hours [20, 86].

Liposomal drugs prolong circulation and tissue delivery half-life with reduced toxicity but still clear predominantly by hepatobiliary pathways, with limited renal excretion (e.g., AmBisome ≤~20%) [90-93]. Iron oxide formulations (e.g., Feraheme) clear almost exclusively hepatobiliary, with Kupffer cell metabolism and sequestration into iron pools [20, 94]. Liposomes are metabolized by hepatic CYPs (CYP3A1, CYP2C6, CYP1A2), whereas iron oxides stimulate distinct lysosomal pathways; such differences argue against a "universal" nanoplatform or universal anti hepatic sequestration strategy [95-97].

The majority of accounts show widespread hepatic uptake irrespective of material and size; particles <~6 nm are set to avoid the liver and be renally cleared [98]. After portal entry, NPs travel through sinusoids; Kupffer cells ensnare the majority with

potential hepatocyte, LSEC, and stellate cell interactions; hepatocytes are capable of secreting NPs into bile canaliculi (**Figure 3**) [98, 99].

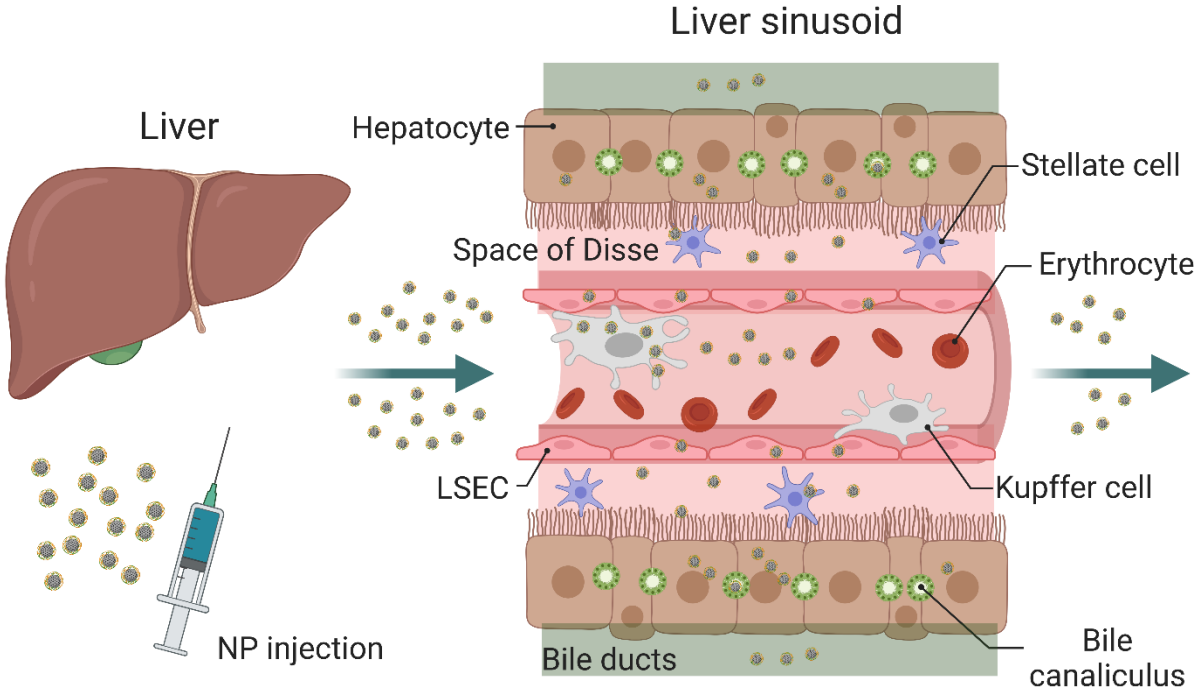


***Figure 3. Schematic of processing and clearance of injected nanoparticles (NPs) by the liver.*** *Injected nanoparticles accumulate in the liver entering via the portal vein. Afterwards, nanoparticles enter the hepatic sinusoid. Kupffer cells engulf the majority of NPs that enter the liver. Such uptake may result into cell death triggered by NPs. Alternatively, NPs may induce Kupffer cell activation leading to the release of proinflammatory cytokines. NPs physicochemical properties affect further distribution, NPs may bypass the space of Disse and be taken up by hepatocytes. Additionally, NPs may interact with liver sinusoidal endothelial cells (LSECs) and/or hepatic stellate cells. After being processed by hepatocytes, NPs may be excreted into the bile duct via bile canaliculi. Created with BioRender.com. Reproduced from ref [11]. Copyright 2023 Elsevier.*

Recirculation can lead to repeated hepatic filtration [74]. **Figure 3** summarizes NP patterns of uptake, metabolism, and excretion.

### 1.4. Key Cellular Players in Nanoparticle Uptake and Processing

Liver cells, hepatocytes (~60%), liver sinusoidal endothelial cells (LSECs), stellate cells, and Kupffer cells (~35% total), and immune subsets (e.g., B cells, endothelial subtypes) internalize and systemically process NPs [74, 87, 100]. While Kupffer cells dominate the uptake, LSECs, stellate cells, and hepatocytes contribute substantially [98, 99]. Quantum dots, for instance, were found in Kupffer cells, B cells, and endothelial cells hours after injection [74]. Trans-endothelial transport is regulated by LSEC fenestrae (50-200 nm) and endocytosis; larger particles than these may be rerouted via the spleen [98, 99]. Lysosomal processing and biliary excretion are hepatocyte-mediated [98, 99]. Oral NP uptake and immune responses are communal to intestinal macrophages and epithelial lineages [98, 99].

Cells of MPS, circulating monocytes and tissue macrophages, scavenge large NP fractions (e.g., ~85% of Kupffer cells, ~25% of splenic macrophages involved) [74]. More than 90% of administered gold NPs and most PLGA NPs have been localized

in Kupffer cells [101]. This creates a paradox: MPS prevents targeted delivery but becomes a useful therapeutic target itself.
Therapeutic implications. In lysosomal storage diseases (LSDs), MPS dysfunction dictates pathology [102, 103]. ERT takes advantage of NP carriers to stabilize enzyme and promote uptake, including CNS targeting through lipid NPs containing β glucocerebrosidase that target CD11b+ cells [104]. Approaches entail receptor targeted enzyme engineering [105, 106] and macrophage-targeted formulations to modulate MPS and prolong NP circulation [107, 108].
In fact, the immune system is broadly targetable. For instance, NPs decorated with ligands to adhesion molecules (ICAM 1, VCAM 1, PECAM 1, selectins) home to inflamed endothelium, reduce leukocyte infiltration, and inhibit inflammation [77, 109, 110]. Targeted antioxidant enzyme carriers (e.g., catalase) show endothelial lung accumulation and inhibition of oxidative stress [77, 109, 110].
Central nervous system (CNS) delivery is limited by the blood-brain barrier (BBB) and related barriers [111, 112]. Solutions include receptor mediated transcytosis, cell penetrating peptides (CPPs), and unconventional pathways (intranasal, intraventricular, intracarotid) [111-113]. Preclinical success (e.g., CPP PLGA for insulin delivery in Alzheimer's models) contrasts with modest clinical translation, a reflection of challenges in selective targeting of brain cell types [111-114].
In general, cells may be divided into (i) high uptake "clearance sinks" (monocytes/macrophages, endothelial cells, hepatocytes, dendritic cells) and (ii) lower volume targets (T/B cells, neutrophils, eosinophils, basophils, erythrocytes, tumor cells, neurons). Both groups are relevant for efficacy, distribution, and toxicity.

### 1.5. Mechanisms of Nanoparticle Uptake and Intracellular Transport

Cells handle nanoscale objects differently compared to micron particles or low-molecular-weight compounds [54]. Two channels of access are dominant: endocytosis and direct membrane fusion, with the latter being more prevalent for most NPs [115, 116]. Protein coronas primarily guide membrane interactions by binding to cell receptors [20, 51-56].
Five main endocytic pathways are involved in NP uptake by cells, namely clathrin mediated endocytosis (CME), fast endophilin mediated endocytosis (FEME), clathrin-independent carrier/glycosylphosphatidylinositol-anchored protein-enriched compartment (CLIC/GEEC) endocytosis, macropinocytosis, and phagocytosis (**Figure 4**) [116]. More recently, an additional non-canonical pathway has been described: cell adhesion molecule (CAM)-mediated endocytosis, in which receptors ICAM-1, VCAM-1, and PECAM-1 mediate uptake [117].
CME forms ~100 nm clathrin coated vesicles; there are receptor dependent and independent versions, and cargo volume can be > 100 nm in some cases [116, 118]. FEME is quick, clathrin independent but dynamin dependent, and forms tubular carriers; its *in vivo* role for NPs is to be determined [116, 119]. CLIC/GEEC is clathrin /dynamin independent, associated with mechanotransduction and specific cargos (glycosylphosphatidylinositol-anchored proteins and hyaluronic acid receptors) [116]. Macropinocytosis internalizes extracellular fluid/particles into >1 μm macropinosomes; highly active in macrophages/dendritic cells [116, 118]. Phagocytosis typically manages >0.5 μm cargo by receptor binding and actin rearrangement but can also internalize small NPs [116, 118]. Caveolae play less notable role than previously estimated under regular physiological conditions [120, 121]. By contrast, CAM-mediated endocytosis (**Figure 4**) has garnered greater attention in the context of endothelial cells in inflammatory or pathological conditions

[117, 122, 123]. Its exact molecular mechanisms are not known, and classical inhibitors of CME or caveolar pathways are not effective, though sensitivity to actin disruption and to certain kinase inhibitors has been observed [117, 124].

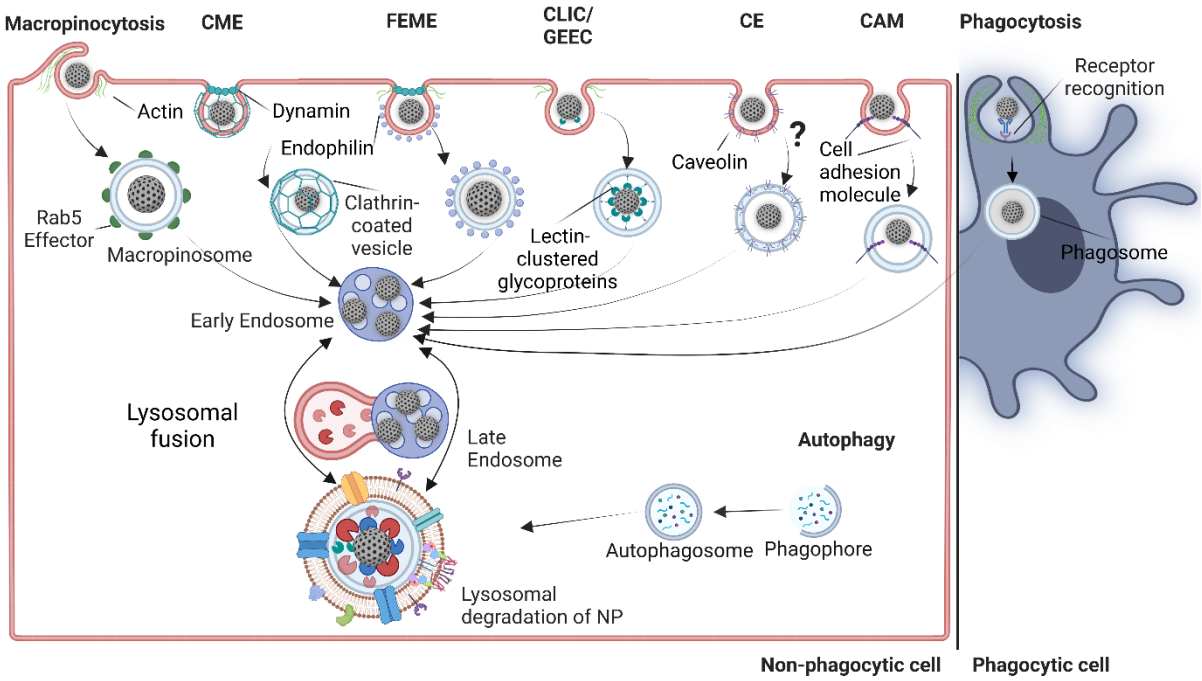


***Figure 4. All roads lead to lysosome.*** *The illustrative scheme depicts all main routes of nanomedicine entry into living cells. Autophagy can be disrupted by lysosomal destabilization induced by NPs. CME – clathrin-coated pit-mediated endocytosis; FEME – fast endophilin-mediated endocytosis; CLIC/GEEC – clathrin-independent carrier/glycosylphosphatidylinositol-anchored protein enriched early endocytic compartment endocytosis, CE – caveolin-dependent endocytosis (current literature shows limited evidence for nanoparticle uptake), CAM – cell adhesion molecule mediated endocytosis. Created with BioRender.com. Reproduced from ref [11]. Copyright 2023 Elsevier.*

Upon entry, vesicles fuse into early endosomes, then traffic to recycling endosomes or mature to late endosomes/lysosomes, dictating degradation or storage [125, 126]. Size, shape, charge, and functionalization of NP dictate routing and can disrupt autophagy or endolysosomal maturation and cause "traffic jams" [127-129].

Diseases resulting from deficits in vesicular trafficking are prevalent; LSDs demonstrate how solitary enzyme deficiencies cause lysosomal malfunction and system wide transport deficiency [130, 131]. NP sequestration can phenocopy aspects of such pathologies, with potential toxicity implications [132-134]. While some carriers are engineered for endosomal release (e.g., PEI "proton sponge," CPPs like TAT) [135, 136], the overwhelming majority of licensed nanomedicines are not; they are instead confined to lysosomes [1, 8, 22, 116, 125, 137-139]. Experimental work consistently shows lysosomal targeting by materials (polystyrene, gold, $TiO_2$, iron oxide, PLGA, lipids) and cell types [140-145]. Notably, mechanisms of in vitro uptake will vary from in vivo, and culture conditions further modulate pathways [116, 146].

***In summary, lysosomes appear to be a common target for many nanoparticles, serving as a critical point for both therapeutic effects and potential toxicity.***

### 1.6. Rationale for Focusing on Lysosomal Pathways as a Central Hub of Nanoparticle Effects

Lysosomes control cellular homeostasis and are primary reservoirs of NP metabolism [116, 125, 137-139]. Cancer cell lysosomes could be structurally compromised and vulnerable to lysosomal membrane permeabilization (LMP), susceptibility that can be addressed by therapies [147, 148]. Since lysosomes interact with inflammation, immunity, neurodegeneration, and oncogenesis, they are targets and effectors of NP activity [147-153]. Methods include lysosome targeted photodynamic therapy and pH responsive carriers releasing payloads in acidic lysosomes; some NPs can induce apoptosis by inducing LMP [154]. Thus, lysosomes are of interest as therapeutic targets and sites for drug delivery, particularly for lysosome related diseases [155].

Lysosomal enzyme or membrane protein defects drive the accumulation of unmetabolized substrate and multisystem disease [150, 156, 157]. ERT has been extensively investigated; NP vehicles (lipid, polymeric, liposomal) have been used to stabilize enzymes, reduce immunogenicity, and improve targeting [158]. Examples include PLGA NPs targeting α 1,4 glucosidase (Pompe disease) [159]; polymeric NPs with α galactosidase A targeting endothelial cells *in vitro/in vivo* [160]; anti ICAM coated NPs targeting enzymes to lysosomes in most organs and facilitating substrate clearance [161]. Chemical targeting moieties (e.g., octadecylrhodamine B on liposomes) and peptide functionalization (e.g., g7 on PLGA) augment lysosomal delivery and restore biochemical defects in models [158, 162]. Albumin NPs and nanoliposomes also stabilize and catalyze enzymatic activity [163]. Though promising, model-to-clinic translation continues to be challenging [158].

To that end, we can summarize the following take-home message. Lysosomes are: (i) passive sinks for the uptake of most clinical NPs; (ii) active decision centers controlling degradation, signaling, and secretion; and (iii) intentional therapeutic targets in oncology, inflammation, and LSDs. ***NP-lysosome interactions are crucial to predicting toxicity, designing safer carriers, and harnessing lysosomal biology for therapy.***

# 2. Aims

This thesis investigates lysosomal pathways as barriers and opportunities in nanomedicine. By examining lysosomal trafficking, degradation, and dysfunction, it aims to bridge the gap between nanoparticle design and biological safety evaluation. Special focus is placed on the influence of lysosomal interactions on nanoparticle performance, with the dual objective of enhancing therapeutic effect and minimizing side effects.

Key determinants of cellular uptake kinetics, lysosomal targeting, and endosomal escape are highlighted for their predictive value in therapeutic modeling. Quantitative understanding of these mechanisms is posited as the central to the rational design of safer, more effective nanomedicines.

Despite advancements in nanoparticle therapy, cytotoxicity is a major obstacle to clinical translation. While toxicity is not specific to nanomedicine, the distinct physicochemical properties of nanoparticles, particularly their ability to cross biological barriers, pose special safety concerns. Emerging evidence suggests that particle size is insufficient to predict biodistribution, underscoring the need for more mechanistic insight. Although there is extensive literature on nanomaterial safety, systematic studies on cellular and molecular mechanisms of toxicity are lacking for

clinically approved nanodrugs. This dissertation addresses this gap by elucidating how nanoparticle uptake, intracellular trafficking, and lysosomal processing impact therapeutic efficacy and possible toxicity.
Specific aims were as follows:

1. Classify prominent types of biomedical nanoparticles and their key physicochemical properties.
2. Elucidate cellular uptake mechanisms and intracellular trafficking.
3. Discuss the lysosome's involvement in nanoparticle fate and function determination.
4. Examine interactions with immune cells and their impact on clearance and immunogenicity.
5. Research nanoparticle-induced toxicity mechanisms, with an emphasis on lysosomal dysfunction and autophagy.
6. Research design strategies for optimizing lysosomal compatibility and reducing adverse effects.
7. Discuss regulatory and translational challenges in nanomedicine clinical development.

Together, these goals provide a comprehensive roadmap to learn how lysosomal biology will impact both the efficacy and safety of nanomedicine.

# 3. Methods

This section summarizes the experimental models, outlines unique methods, and presents key results from methodological studies. Bold numbers in brackets refer to the original publications listed in Section 10.

### 3.1. Nanoparticle Purchase and Synthesis

For the exploration of nanoparticle (NP) uptake, trafficking, and cellular functional impact, a library of versatile NPs was prepped involving both commercially procured and synthon-derived materials. This allowed scope for analysis of size, shape, surface chemistry, and biofunctionality on cellular interaction.

*Commercial Nanoparticles*

Clinically approved superparamagnetic iron oxide nanoparticles (SPIONs) like Resovist™ (Bayer Schering) and Supravist™ (kindly donated by Prof. V. Rasche) were used as material controls. Their hydrodynamic diameter, zeta potential, and surface charge were measured using dynamic light scattering (DLS) in saline. Fluorescence-labeled SPIONs (MicroMod, Germany) and core-shell analogues (Chemicell GmbH, Berlin) were applied as alternative material for comparison experiments (**10.1; 10.2; 10.3; 10.8; 10.9; 10.12; 10.13**).

*Fluorescent polystyrene nanoparticles* were synthesized via miniemulsion polymerization. Surfactants were removed via ultrafiltration, dialysis, and washing. The final particles were analyzed via UV-Vis spectroscopy, DLS, and zeta potential measurements to ensure stability and suitability for model investigations (**10.4; 10.6**).

*PLL-Coated SPIONs*

SPIONs with a poly-L-lysine (PLL) coating yielded positively charged nanoparticles. TEM showed 6.2 nm cores with a hydrodynamic diameter of ~141 nm. Functionalization was confirmed using FTIR spectroscopy, and saturation magnetization was determined via SQUID (**10.7**).

*Morphologically Controlled Iron Oxide Nanoparticles*

Iron oxide nanocubes (IO-cubes) and clusters (IO-clusters) were synthesized through thermally decomposing specially prepared solvent and heating protocols. Hydrophobic nanoparticles were rendered water-dispersible by PEGylation using EDC/NHS coupling. PEG-conjugated nanostructures were labeled with Cy5 and purified for biological assays (**10.17**).

*DNA Nanostructures*

Six-helix bundle (6HB) DNA nanostructures were built using PAGE-purified oligonucleotides (IDT), with one strand labeled with AlexaFluor-488. Structures were annealed in $Mg^{2+}$-based buffer and verified by electrophoresis and DLS. Bioactive peptide coatings (K10, aurein-derived) were synthesized by Fmoc solid-phase synthesis and verified by MALDI-TOF. Peptide-functionalized DNA nanostructures enabled mechanistic studies on lysosomal interference and immune signaling (**10.19; 10.24; 10.25**).

### 3.2. Nanoparticle Physicochemical Characterization

Physicochemical analysis included DLS-measurement of hydrodynamic diameter, zeta potential, and polydispersity in physiological media. TEM (JEOL JEM-1400) was employed to achieve morphological data, where ≥500 particles per sample were counted using ImageJ (**10.17**).

X-ray diffraction (XRD) patterns of IO-cubes and clusters were recorded using Co Kα radiation to evaluate crystallinity. Magnetometry (VSM) was done to study magnetic properties (**10.17**).

Peptide-functionalized DNA nanostructures were assessed by gel electrophoresis, AFM (Bruker Multimode 8), and FRET-based melting analysis to ascertain structural stability (**10.19; 10.24; 10.25**).

### 3.3. Cell Culture

Various human cell lines were used, including hepatic (HepG2, Huh7, Alexander), glioblastoma (U87MG), and kidney-derived HEK293T cells. Monocyte-derived human macrophages were generated from buffy coats and differentiated using M-CSF (**10.1; 10.2; 10.3; 10.11; 10.19; 10.24; 10.25**).

THP-1 monocytic cells were differentiated into macrophages using PMA. Cell authenticity was authenticated by STR profiling and Mycoplasma screening (**10.4; 10.6**).

Cells were plated into appropriate formats (96- or 24-well plates) at 5,000–50,000 cells/well, as required for assays.

### 3.4. Animal Models

Following models were used.

*Mouse systemic NP administration*: NMRI mice received intravenous SPIONs (10 µmol Fe/kg) with or without pretreatment with the antioxidant edaravone. MRI was performed in a 3T system and tissue was collected for histology (**10.2**).

*Chick chorioallantoic membrane (CAM) tumor model*: PC-3 prostate cancer cells were seeded onto the fertilized egg's chorioallantoic membrane. NPs were injected intravenously, and tumors were analyzed for macrophage invasion using KUL01 staining (**10.4**).

*Rat spinal cord injury (SCI) model*: T8-T9 compression injury was induced by an inflatable balloon catheter. Labeled mesenchymal stem cells were transplanted and later analyzed for survival and delivery (**10.7**).

All protocols using animals conformed to ethical standards and institutional guidelines.

### 3.5. Imaging Techniques

Magnetic resonance imaging (MRI) was used for in vivo tracing of NP distribution in mice using a 3T platform and animal-specific coils (**10.2**).
Spinning disk systems (IXplore SpinSR, Olympus) with 405/488/561 nm excitation lasers and dual-camera acquisition were used for Confocal Microscopy (**10.9; 10.11; 10.12; 10.13; 10.15; 10.19; 10.24; 10.25**).
Super-Resolution Microscopy was used for high-resolution imaging of lysosomal organization in cells that had been treated with DNA nanostructures (**10.13; 10.24**).
Transmission electron microscopy (TEM) was used for ultrastructural visualization of NP uptake in macrophages. The cells were fixed, osmium tetroxide post-fixation, resin embedding, sectioning, staining, and imaging (**10.1; 10.2; 10.3**).

### 3.6. Histology and Immunohistochemistry

Tissue sections (5 µm) were iron-stained with Prussian Blue and for apoptosis with caspase-3 immunofluorescence with Hoechst counterstaining (**10.2**).
In the rat SCI model, spinal cords were cryosectioned and analyzed for GFP-labeled MSCs. Quantification was obtained based on corrected total cell fluorescence (CTCF) parameters in ImageJ (**10.7**).

### 3.7. Functional Cellular Assays

*Viability Assays*
Vitality was ascertained through alamarBlue or XTT assays. Membrane integrity was studied through propidium iodide (PI) staining. Apoptosis was established by caspase-3 activity and Annexin V/PI staining by microscopy and flow cytometry (**10.9; 10.11; 10.12; 10.13; 10.15; 10.18; 10.19; 10.24; 10.25**).
*Mitochondrial Health*
JC-1 dye was used to assess mitochondrial membrane potential. Red/green fluorescence ratio indicated polarization status. ROS formation was evaluated by MitoSOX Red and cumulative ROS using flow cytometry (**10.9; 10.11; 10.12; 10.13; 10.15; 10.18; 10.19; 10.24; 10.25**).
*Lysosomal Function*
Acridine orange and LysoTracker Red were employed to quantify lysosomal membrane integrity, while LysoSensor Blue was used to quantify pH. Measurements were taken using plate reader and confocal microscopy and quantified by ImageJ (**10.9; 10.11; 10.12; 10.13; 10.15; 10.18; 10.19; 10.24; 10.25**).
*Oxidative Stress*
Total intracellular ROS and superoxide levels were quantified using Abcam's Cellular ROS Detection Kit and were observed by microscopy or flow cytometry (**10.9; 10.11; 10.12; 10.13; 10.15; 10.18; 10.19; 10.24; 10.25**).

### 3.8. Particle-Protein Interactions

Nanoparticles were incubated in serum-containing medium to examine protein corona formation. Particles were washed following incubation, and adsorbed proteins were analyzed by SDS-PAGE and Coomassie staining (**10.4; 10.19**).
Fluorescence Correlation Spectroscopy (FCS) was used to detect corona-induced changes in diffusivity. The measurements were obtained using a confocal microscope equipped with a 470 nm laser and single-photon detectors. Data were

fitted to a 3D diffusion model, and hydrodynamic radius changes were used as measures of protein adsorption (**10.19**).

### 3.9. Generation of 3D Multicellular Cancer Cell Aggregates

Three-dimensional cancer spheroids were generated in ultra-low attachment 96-well plates (BIOFLOAT™). Hepatic and glioblastoma cells were seeded at 20,000 cells/well and left to incubate for 3 days.
48 hours was spent incubating spheroids with nanoparticles. Viability was assessed by PI and Hoechst staining, with confocal imaging, offering a physiologically relevant model for NP interaction studies (**10.12; 10.24**).

### 3.10. Statistical Analysis

All results are presented as mean ± SEM. One-way ANOVA followed by Newman-Keuls post hoc test by MaxStat Pro 3.6 was employed to find statistical distinctions. Significance was considered at $P < 0.05$.
For image-based assays, over 30 cells/condition were considered in three independent experiments. ImageJ was utilized to measure the fluorescence intensity, colocalization, lysosomal acidity, and morphology according to a well-established confocal imaging protocol.

## 4. Results

Engineered nanoparticles are now revolutionary tools in modern biomedicine for drug delivery, diagnostics, and regenerative therapies (**10.21; 10.27**). While possessing tremendous potential, their clinical translation is limited by a lack of targeting specificity, unclear biological interactions, and toxicity concerns (**10.21; 10.27**). One of the key players in these interactions is the lysosome, which is not only tasked with digesting internalized nanoparticles but also a potential site of toxicity upon disruption (**10.14; 10.21; 10.27**).
This chapter summarizes experimental findings exploring nanoparticle uptake, lysosomal trafficking, and biological fates in diverse cell models: macrophages, hepatocellular lines, mesenchymal stem cells, and monocytes. A wide range of nanoparticle types were investigated: superparamagnetic iron oxide nanoparticles (SPIONs), polystyrene nanospheres, magnetite nanocubes/clusters, and DNA nanostructures (DNs). Across these systems, a unifying theme emerged: the lysosome as a central decision-making organelle where nanoparticle trafficking, protein corona remodeling, and cell stress responses intersect.

### 4.1. Uptake and Intracellular Distribution

*Determinants of Uptake*
Nanoparticle internalization was invariably controlled by physicochemical characteristics like size, surface charge, and surface coating, in addition to the presence of a protein corona. SPIONs and ultrasmall SPIONs (USPIOs), for example, were internalized quickly by human macrophages through scavenger receptor A- and clathrin-mediated endocytosis, as verified through inhibitor studies and electron microscopy (**10.1; 10.2; 10.3; 10.5**). SPIONs were internalized more effectively by macrophages compared to USPIOs, with ~10-fold greater iron uptake in SPION-treated cells (**10.1; 10.2; 10.3**). Interestingly, the type of endocytic pathway varied with particle composition and surface functionalization. For example, amino-functionalized polystyrene nanoparticles (PS-$NH_2$) were internalized more efficiently compared to carboxylated PS-COOH (**10.4; 10.8**). PS-$NH_2$ particles were

internalized primarily through macropinocytosis, whereas PS-COOH uptake was mostly clathrin- and dynamin-dependent. Clathrin genetic knockdown confirmed its significant contribution in both cases, wherein ~70% reduction in particle uptake was noted (**10.4**).

*Cell-Type Variability*

Uptake kinetics varied among cell types. Hepatocellular carcinoma cell lines (HepG2, Huh7, Alexander) showed time- and dose-dependent SPION uptake to saturation 12 hours post-exposure (**10.13**). DNA nanostructures (6-helix bundles) showed less uptake due to their high negative charge and instability in serum-containing media but were taken up over time, with the amounts of uptake highly correlating with cell size, a novel finding that points to the cell surface area as a key modulator of nanoparticle uptake (**10.19**).

*Role of the Protein Corona*

Protein corona formation significantly affected uptake behavior. If DNs or polystyrene particles were pre-incubated with fetal bovine serum (FBS), the uptake efficiency was considerably decreased in the majority of cell types, especially in hepatic cells (**10.19**). Confocal imaging revealed that corona-coated particles exhibited delayed entry into lysosomes, while macrophages, which naturally have high scavenger receptor expression levels, were less affected by corona formation (**10.3; 10.4; 10.13; 10.19**). This highlights the significant role of the extracellular environment in regulating nanoparticle-cell interactions.

*Intracellular Fate and Localization*

After uptake, most nanoparticles trafficked to lysosomes and endosomes, marked by co-localization with the LAMP1 and LysoTracker markers (**10.3; 10.4; 10.13; 10.19**). SPIONs trafficked to lysosomes after 6-12 hours, consistent with their clinical application in imaging studies (**10.1; 10.2; 10.3; 10.13; 10.16**). Polystyrene nanoparticles showed delayed and, at times, incomplete trafficking to lysosomes, with nanoparticles remaining in endosomes even after 48 hours (**10.4; 10.6**). Surface-modified DNA nanostructures, and in particular those coated with membrane-active peptides like aurein, showed partial endosomal escape but also induced lysosomal destabilization (**10.19; 10.24**).

Summarizing, one can conclude that nanoparticle uptake and trafficking are driven by a complex interplay between particle properties, cell phenotype, and extracellular determinants such as the protein corona. Additionally, surface functionalization can redirect intracellular trafficking, determining whether particles are sequestered, escape into the cytosol, or trigger stress responses.

### 4.2. Lysosomal Fate and Dysfunction

Independent of their material composition or surface chemistry, all nanoparticles tested accumulated in lysosomal vesicles following endocytic uptake. This convergence was confirmed by co-localization studies with LysoTracker, LAMP1, and electron microscopy (**10.1; 10.2; 10.3; 10.10; 10.12; 10.13; 10.16; 10.17**). These tests demonstrated that various particles, including SPIONs, polystyrene beads, magnetite nanoclusters, and DNs, were all contained within acidic vesicular compartments, placing the lysosome as a universal focal point for nanoparticle destiny (**10.1; 10.2; 10.3; 10.10; 10.12; 10.13; 10.16; 10.17; 10.24; 10.25**).

Despite this shared fate, the impact of lysosomal accumulation was fairly divergent depending on the specific nature of the nanoparticles. For example, dextran coated SPIONs and USPIOs, though trafficked to lysosomes with rapid efficiency, did not induce noticeable structural damage after 24 hours (**10.1; 10.2; 10.3; 10.9 10.10;**

**10.12; 10.13; 10.16)**. Transmission electron microscopy revealed intact lysosomal membranes with iron clusters, consistent with their proven biocompatibility and clinical imaging use (**10.1; 10.2; 10.3**).
In contrast, positively charged PS-$NH_2$ polystyrene particles caused intense lysosomal swelling, vacuolization, and membrane permeabilization (**10.4; 10.6**). These effects were expressed by acridine orange staining and cytosolic release of cathepsin B, highlighting the disruptive impact of cationic surface charge on the integrity of lysosomes (**10.4; 10.6**). Magnetite nanocubes and nanoclusters, although internalized more slowly than PS-$NH_2$, induced lysosomal stress in a time-dependent manner. Effects were more pronounced after 48 to 72 hours of incubation, suggesting that the morphology of iron oxide crystals also plays a role in disrupting lysosomal structure over time (**10.17**).
DNs offered an unprecedented platform to examine the influence of surface design on lysosomal behavior (**10.19; 10.24; 10.25**). Unmodified DNs underwent expected lysosomal degradation, due to their sensitivity to nucleases. In contrast, surface-modified DNs showed divergent effects. Decalysine-coated DNs (K10-DNs) induced lysosomal acidification and showed increased localization in LAMP1-positive compartments, suggesting improved retention and sequestration potential for therapy (**10.19; 10.24; 10.25**). Aurein-coated DNs induced lysosomal alkalinization and swelling, attributed to the aurein peptide's amphipathic and pore-forming nature. These particles showed reduced lysosomal co-localization over time, suggesting partial escape from endolysosomal compartments to the cytosol (**10.19; 10.24; 10.25**). These findings emphasize that lysosomal trafficking and stress can be intentionally controlled by nanoparticle surface functionalization with the ability to either favor lysosomal retention or facilitate cytosolic delivery.
The degree of LMP also varied by nanoparticle type. PS-$NH_2$ particles induced fast and complete LMP at 24 hours (**10.4; 10.6**), while iron oxide nanocubes caused a slower, progressive destabilization (**10.17**). Aurein-coated DNs displayed a more selective profile, triggering LMP in a subpopulation of lysosomes (**10.19; 10.24; 10.25**). Interestingly, lysosome-disrupting nanoparticles also influenced autophagic flux (**10.13; 10.17**). For instance, magnetite nanocluster treatment increased LC3B-II levels and puncta formation, indicative of autophagosome accumulation and impaired lysosomal degradation (**10.13; 10.17**), a known cellular stress response pathway.
Cell type also influenced lysosomal responses. Macrophages, owing to their innate scavenger function, took up high nanoparticle loads but tolerated higher levels before LMP (**10.1; 10.2; 10.3; 10.10; 10.12; 10.13; 10.16; 10.17; 10.24; 10.25**). Hepatocellular carcinoma cells, however, particularly when metabolically stressed in the form of pre-treatment with palmitic acid, were more susceptible to lysosomal destabilization. That such external metabolic status interacted with nanoparticle exposure highlights the necessity of cellular context in determining lysosomal susceptibility (**10.1; 10.2; 10.3; 10.10; 10.12; 10.13; 10.16; 10.17; 10.24; 10.25**).
Briefly, lysosomes are central nanoparticle processing centers, but their composition and activity are controlled by particle-dependent determinants such as surface coating, shape, and charge. DNs demonstrate how lysosomal fate can be programmatically determined, some coatings enable acidification and retention, while others allow for destabilization and escape. Ultimately, lysosomal stress can induce more widespread cellular responses, including defective autophagy and apoptosis. These effects are further controlled by both extrinsic natural conditions,

for instance, protein corona formation and metabolic stress, and intrinsic cellular properties such as cell type.

### 4.3. Inflammasome Activation and Immune Responses

While lysosomal sequestration was a common fate for the majority of nanoparticle systems, the biological consequence following this trafficking differed widely according to the surface chemistry and structural attributes of the particle. A theme common amongst systems was that nanoparticles possessing lysosome-disrupting potential would activate innate immune pathways, most prominently the NLRP3 inflammasome. This cytosolic multi-protein complex is a sensor of cellular stress and responds to signals including lysosomal compromise and oxidative stress.

Among the nanoparticles tested, amino-functionalized polystyrene particles (PS-$NH_2$) were the most potent inflammasome activators (**10.4; 10.6**). In human macrophages, these particles induced lysosomal disruption, cytosolic release of cathepsin B, and mitochondrial damage. This was followed by caspase-1 activation and release of mature IL-1β, a signature pro-inflammatory cytokine. These responses were absent in cells treated with carboxylated PS-COOH particles, suggesting that surface chemistry is a critical determinant of inflammasome activation (**10.4; 10.6**).

The NLRP3 inflammasome's role at the core of this process was confirmed in gene-silencing studies. NLRP3 or ASC (inflammasome adaptor protein) knockdown abolished caspase-1 activation and IL-1β release, confirming pathway specificity. Importantly, while IL-1β was robustly secreted, TNF-α levels remained unchanged, showing that immune response was uniquely linked to inflammasome activation rather than to general pro-inflammatory signaling (**10.4; 10.6**).

Further research discovered that mitochondrial reactive oxygen species (ROS) generation played a parallel role in inflammasome promotion. Macrophages treated with PS-$NH_2$ nanoparticles displayed heightened mitochondrial superoxide, as measured by MitoSOX staining (**10.4; 10.6**). Pre-treatment with N-acetyl cysteine (NAC), a scavenger of ROS, significantly suppressed IL-1β release and caspase-1 cleavage, providing strong evidence that oxidative stress and lysosomal disruption in tandem trigger the inflammasome cascade. Molecularly, oxidative stress was discovered to liberate thioredoxin-interacting protein (TXNIP) from thioredoxin, where it is free to bind NLRP3 and promote inflammasome assembly (**10.4; 10.6**).

While macrophages mounted strong inflammasome-mediated response, hepatocellular carcinoma cells mounted more covert responses. The cells released reduced levels of IL-1β but responded to DNA nanostructure treatment with increased transcription of interferon-stimulated genes and JAK-STAT activation. Interestingly, the response was nanoparticle surface property-dependent: decalysine-functionalized DNA nanostructures, which preserved lysosomal integrity, caused minimal immune activation, whereas aurein-functionalized constructs, which promoted lysosomal destabilization, potently upregulated interferon-related gene expression(**10.19; 10.24; 10.25**).

These results demonstrate that nanoparticles trigger immune responses through multiple, converging mechanisms. In professional immune cells like macrophages, lysosomal destabilization and ROS production collaborate to initiate inflammasome signaling. In non-immune cells like hepatocytes, nanoparticle exposure can trigger more general inflammatory programs, especially in the context of metabolic stress. Collectively, these accounts highlight how nanoparticle surface design dictates not

only cellular uptake and fate but also the extent and specificity of the immune response.

### 4.4. Cytotoxicity and Cell Death Pathways

The extent and pathway of nanoparticle-induced cytotoxicity were extremely variable as a function of material composition, surface chemistry, particle morphology, and cell type. Clinically used dextran-coated SPIONs and USPIOs were tolerated well at short durations and at the doses commonly used in diagnostic imaging (**10.13**). Following prolonged exposure, however, revealed evidence of toxicity, particularly in macrophages, that had ingested high iron loads (**10.1; 10.2**). After five days of treatment, both SPIONs and USPIOs decreased cell viability dose-dependently, with greater cytotoxicity of the USPIOs compared to SPIONs (**10.1; 10.2**). Early apoptotic markers like exposure of phosphatidylserine were detected after three days, and caspase-3 activation confirmed apoptotic death, particularly with USPIOs (**10.1; 10.2**).

Synthetic particles such as amino-functionalized polystyrene spheres, iron oxide nanoclusters and nanocubes, and peptide-coated DNs, however, exhibited significantly greater cytotoxicity (**10.6; 10.8; 10.17; 10.24**). These nanoparticles induced time- and concentration-dependent cytotoxicity to both immune and parenchymal cells. For example, polystyrene PS-$NH_2$ nanoparticles and magnetite nanoclusters triggered early apoptotic events such as annexin V binding and cleavage of caspase-3. Cells treated with these nanoparticles displayed typical apoptotic morphologies such as nuclear fragmentation, cytoplasmic blebbing, and mitochondrial damage (**10.6; 10.8; 10.17; 10.24**).

Hepatocellular carcinoma cell lines (HepG2, Huh7, and Alexander) showed variable response to nanoparticle treatment. Alexander cells were most sensitive, as predicted from their decreased expression of the anti-apoptotic protein Bcl-2 (**10.12; 10.13; 10.17; 10.24**). Mitochondrial depolarization was an invariable early event, shown by a redistribution of JC-1 dye fluorescence (**10.12; 10.13; 10.17; 10.24**). This perturbation of mitochondrial membrane potential was followed by enhanced mitochondrial ROS generation and release of cytochrome c to the cytosol, events that form the intrinsic apoptosis pathway (**10.12; 10.13; 10.17; 10.22; 10.24**).

The oxidative stress that caused mitochondrial damage was directly linked to nanoparticle properties. MitoSOX staining confirmed increased mitochondrial superoxide after treatment with PS-$NH_2$ and iron oxide nanoclusters (**10.6; 10.12; 10.17**). These effects were partially reversible with antioxidant treatment, indicating ROS production as a major driving force behind cell death. Downstream, caspase-mediated apoptosis was corroborated by immunoblotting detection of cleaved caspase-3 and associated substrates (**10.6; 10.12; 10.17**).

Notably, apoptosis was only one of the pathways of death that were observed. Lysosomal membrane permeabilization, which is a typical result of nanoparticle treatment, triggered other, caspase-independent pathways (**10.1; 10.2**). Over-spill of lysosomal enzymes, and notably cathepsins, into the cytoplasm triggered apoptosis-like features that could persist even after the caspases were not activated. This pathway of death by lysosomes was most prominently evident in macrophages treated with PS-$NH_2$ particles and hepatocytes treated with aurein-functionalized DNA nanostructures (**10.6; 10.12; 10.17; 10.24**). Inhibition of cathepsin B reduced death in cells, confirming its biological function in these processes.

Cell stress responses were also amplified under pathological conditions. Under hepatocellular carcinoma cells pre-conditioned with palmitic acid to mimic a condition

of steatosis, iron oxide nanoparticle exposure generated synergistic toxicity (**10.22**). In the model, destabilization of the lysosome cooperated with endoplasmic reticulum (ER) stress, as indicated by BiP and CHOP upregulation (**10.22**). Nuclear translocation of NF-κB and increased apoptosis followed, suggesting that pre-existing metabolic stress can reduce the threshold for particle-induced death (**10.22**).
Cell type again was the determining factor. Macrophages exhibited high levels of nanoparticle uptake but were viable for longer durations, likely as a consequence of their inherent process of clearance of foreign particles (**10.1; 10.2**). Nevertheless, they too succumbed eventually to oxidative and lysosomal damage with prolonged exposure. Cancer cells, on the other hand, exhibited more rapid apoptotic responses but varied considerably between lines (**10.12; 10.13; 10.17; 10.22; 10.24**). Therefore, for instance, HepG2 cells were very resistant, while Alexander cells showed robust caspase induction and mitochondrial collapse (**10.12; 10.13; 10.17; 10.22; 10.24**). In the same population of cells, even heterogeneity was observed, particularly in glioblastoma models, where there was survival of some subpopulations but apoptosis of others (**10.12; 10.13; 10.17; 10.22; 10.24**).
Surface modification had a significant effect on cytotoxic activity. Decalysine-coated DNs triggered lysosomal acidification and were nontoxic, whereas aurein-coated DNs triggered lysosomal membrane disruption and apoptosis (**10.12; 10.13; 10.17; 10.22; 10.24**). These findings highlight the importance of surface chemistry not just in uptake and trafficking but also in determining whether cells survive or provoke programmed death pathways (**10.12; 10.13; 10.17; 10.22; 10.24**).
In summary, nanoparticle cytotoxicity results from many, often crossing pathways. Mitochondrial damage and ROS production are crucial features, with lysosomal breakdown and cathepsin release contributing additional apoptotic stress. ER stress and cellular vulnerabilities also influence the outcomes. The findings confirm that nanoparticles are not inert carriers but active biological agents where design plays a direct role in dictating cellular fate.

### 4.5. In Vivo and Translational Insights

To translate *in vitro* observations to physiological systems, several in vivo models were utilized to evaluate nanoparticle biodistribution, clearance, cytotoxicity, and functional application. Commercial dextran-coated SPIONs were injected intravenously into mice and demonstrated rapid uptake by the liver and spleen as would be expected of mononuclear phagocyte system clearance (**10.1; 10.2**). Iron staining confirmed localization within Kupffer cells, liver-resident macrophages, confirming their key function in nanoparticle clearance (**10.1; 10.2**).
Even though macrophage uptake of nanoparticles was not inert, nanoparticle-treated Kupffer cells had clear signs of apoptosis and stress after five days' treatment. Activated caspase-3 co-localized exactly with iron-positive Kupffer cells, and liver macrophage numbers fell by a large proportion after a single injection (**10.1; 10.2**). Significantly, injection of the antioxidant edaravone blocked SPION-induced apoptosis without affecting iron uptake, demonstrating that ROS played a causal role *in vivo*, as it did *in vitro* (**10.1; 10.2**).
Nanoparticle biodistribution was also evaluated in a chick chorioallantoic membrane (CAM) tumor model with fluorescently tagged polystyrene nanoparticles (**10.4**). Amino-functionalized PS-$NH_2$ and carboxylated PS-COOH particles exhibited the targeting preference to the liver and tumor xenografts, respectively (**10.4**). Macrophages in both tissues internalized primarily the nanoparticles, which demonstrates that macrophage-mediated uptake is the cause of biodistribution not

only in detoxification organs like the liver but also in the tumor microenvironment (**10.4**).
In addition to passive targeting, nanoparticles were also used as therapeutic delivery functional tools. Magnetic targeting of SPION-labeled mesenchymal stem cells (MSCs) was highly efficient in localizing and retaining cells at the site of lesion in a rat spinal cord injury (SCI) model (**10.7**). Of note, MSCs transmigrated from cerebrospinal fluid to injured spinal tissue under magnetic guidance, critically a prerequisite for successful regeneration (**10.7**). Efficiency of delivery was threefold higher with SPION-labeled cells under magnetic guidance compared with unlabeled controls or magnetically unguided cells (**10.7**).
Immune responses were still a limitation, however. Infiltrating macrophages targeted SPION-labeled MSCs within the injury site actively at the cost of long-term cell survival (**10.7**). This finding underscores the need to couple magnetic targeting approaches with immunomodulatory approaches in the prevention of premature clearance or inflammation-mediated rejection (**10.7**).
Collectively, these *in vivo* studies validated several of the mechanistic findings from the in vitro research. Macrophages consistently were the primary controllers of nanoparticle fate, dictating clearance and local immune responses. Lysosomal sequestration continued to be the most common intracellular site, particularly in macrophages. Within tumor and injury models, lysosomal stress responses appeared to govern both cytotoxicity as well as the immune microenvironment at nanoparticle-treated sites.
Furthermore, these studies showed that design of the surface dictates both utility and safety. SPIONs demonstrated how rational design can achieve clinical translation by balancing imaging performance with tolerability. Morphologically distinct iron oxide nanoclusters or polystyrene nanoparticles, however, caused lysosomal damage and inflammation that may be utilized therapeutically in cancer, but must be stringently regulated. DNA nanostructures, though not yet tested *in vivo* here, demonstrate how it might be possible to use programmable surfaces to control immune activation or deliver targeted release in future translation applications (**10.20; 10.26**).
Lastly, the physiological environment, spanning from cell type to tissue microenvironment, significantly affects nanoparticle behavior (**10.23**). Even clearly defined particles behave differently between systems as a function of local macrophage density, metabolic stress, and immune status (**10.23**). These observations underscore that successful clinical delivery of nanoparticles depends on a systems-level understanding of how materials interact with different biological milieus. Only by taking this complexity into account can nanoparticles be safely designed for effective biomedical use.

# 5. Conclusions

This thesis documents a set of experiments with different types of nanoparticles, mammalian cells, and *in vivo* models, all converging to a unifying theme: ***the lysosome is the decision-making organelle determining nanoparticle fate and function***.
Regardless of core material, superparamagnetic iron oxide, polystyrene, or DNA nanostructures, nanoparticles were taken up by receptor-mediated endocytosis, macropinocytosis, or phagocytosis and trafficked to the endo-lysosomal system as a default route. Within lysosomes, nanoparticle destinies ranged from inert sequestration to profound cytotoxicity, depending on their surface chemistry,

morphology, and protein corona. Dextran coatings, for example, promoted short-term lysosomal compatibility, while cationic surfaces and nanoclusters caused lysosomal swelling and bursting.
Cellular environment conditioned such responses: macrophages were able to tolerate higher particle burdens due to their phagocytic role, while hepatocytes and tumor cells (e.g., Alexander hepatocellular carcinoma) were more sensitive to lysosomal stress. Lysosomal destabilization initiated downstream stress responses, cathepsin release, inflammasome activation, ER stress, and mitochondrial dysfunction, resulting in apoptosis or necrosis.
The lysosomal dysfunction was rarely an isolated event; it was strongly correlated with mitochondrial and ER stress, suggesting an interconnected organelle response to nanoparticle-induced perturbations. The creation of a protein corona added another level of complexity, sometimes reducing surface reactivity but also inhibiting therapeutic effect.
An integrated mechanistic framework is unmasked:

- Nanoparticle uptake is physicochemical property-governed and protein corona-typed.
- Lysosomal trafficking is default and subsequent events depend on particle design and cell type.
- Biocompatible particles (e.g., PEG-, dextran-coated) are inertly sequestered; reactive surfaces lead to disruption of lysosomal integrity and induce systemic stress.
- The cellular response is intrinsic (cell metabolism, phenotype) and extrinsic (particle load, corona, morphology).

*In vivo*, macrophages became reservoirs for the nanoparticles. Immune infiltration and apoptosis in the tumors and lysosome-compatible SPIONs enabled magnetic cell targeting in spinal cord injury models, which were safe.
This study identifies the lysosome as a central regulator of nanoparticle-cell interactions. It is neither an endpoint degradative compartment nor a sensor and signaling center that interprets nanoparticle identity and reports biological outcomes, from tolerance to toxicity. This principle provides a framework for more predictive nanotoxicology and rational design of next-generation nanomedicines.

# 6. Future Perspectives: Predictive Modelling of Nanodrug Cytotoxicity

This dissertation categorizes lysosomal compromise, along with oxidative stress and mitochondrial injury, as the primary cause of nanodrug cytotoxicity. Interplay among organelles exacerbates cell injury, and this emphasizes the importance of lysosomal integrity while designing nanodrugs.
Based on these results, our objective was to create predictive models for the design of safer nanodrugs. We combined experimental information with an AI-enhanced literature review, centered on clinically relevant nanodrugs (**10.27**). By using the Elicit platform, we mined and compared cytotoxic mechanisms across nanodrug classes and developed critical mechanisms such as inflammasome activation, lysosomal destabilization, ROS generation, and inhibition of autophagy.
A qualitative comparative matrix (**Table 1**) was constructed, ranking nanodrug classes (e.g., liposomes, polymer conjugates, metal NPs) by dominant toxic effects. Mechanisms were ranked—strong, moderate, or weak, based on frequency of

reports. While quantitative data are scant, this matrix presents a structure-based, mechanism-oriented toxicity overview.

The toxicological profiles of nanodrugs depend on material class, administration route, and physicochemical properties, particularly size, charge, coating, and shape (**Table 1**). Notably, smaller nanoparticles (<15 nm) show greater toxicity due to higher surface area and cellular interaction potential (**10.27**). Positive surface charges increase cytotoxicity via membrane disruption, while surface modifications (e.g., PEGylation) can mitigate or alter these effects (**10.27**). The protein corona also modulates immune recognition and uptake.

***Table 1.*** *Qualitative comparative matrix showing the relationship between nanodrug types and their predominant cytotoxic mechanisms. Reproduced from ref [3]. CC BY 4.0.*

| Nanodrug Category | Cytotoxic Trigger | | | | | Ref. |
|---|---|---|---|---|---|---|
| | Oxidative Stress | Lysosomal Dysfunction | Membrane Disruption | Inflammatory Response | Mitochondrial Dysfunction | |
| Liposomes | ++ | + | +++ | ++ | + | [164] |
| Metal NPs (Au, Ag) | +++ | ++ | +++ | ++ | ++ | [165] |
| Quantum dots | +++ | ++ | + | ++ | +++ | [166] |
| Carbon nanotubes | ++ | + | ++ | +++ | ++ | [167] |
| Polymeric NPs | + | +++ | ++ | + | + | [168] |
| Iron oxide NPs | +++ | + | ++ | ++ | ++ | [169] |
| Silica NPs | ++ | ++ | +++ | + | + | [170] |

+++ Strong association (frequently reported/primary mechanism); ++ Moderate association (commonly reported); + Weak association (occasionally reported).

To quantify risk, we developed a *Toxicity Index Scoring System* (**Figure 5**), assigning scores (0–21) based on nanoparticle attributes (e.g., size, shape, charge), cellular effects (e.g., ROS, cytokines), and modifications (e.g., PEGylation). This allows categorization into low, moderate, or high toxicity risk and serves as a preclinical decision-making tool (**10.27**).

In parallel, we define a *Cytotoxicity Checklist* (**10.27**), following the successful MIRIBEL guidelines [171], but tailored to address the specific needs of minimizing nanodrug toxicity. Although this checklist is itself a temporary tool and not completely specific for the wide variety of nanoparticle types, it is a considerable advancement toward nanotoxicology reporting standardization and reproducibility (**10.27**).

This checklist shows pragmatic design principles: active targeting preference to passive targeting to ensure maximum selectivity; physicochemical design (e.g., >15 nm diameter, neutral or negative surface charge, spherical shape); biocompatible coatings deposited; evading previously established cytotoxic initiators such as oxidative stress and lysosomal disruption; and inclusion of evidence-based risk scoring algorithms like the *Toxicity Index* (**Figure 5**) (**10.27**). It also encourages mechanistic confirmation and incorporation of material-specific dangers at the nanoparticle design level.

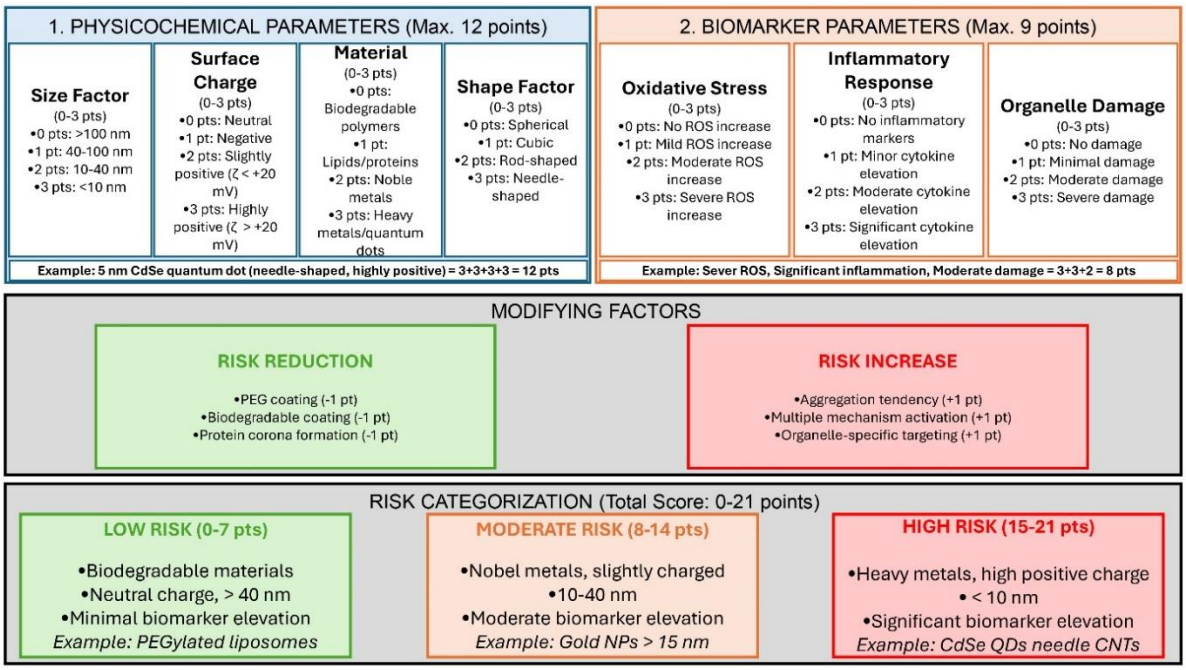


***Figure 5. Proposed Toxicity Index Scoring System.*** *QDs: quantum dots; NPs: nanoparticles; CNTs: carbon nanotubes; pt/pts: point/points. Created with BioRender. Reproduced from ref [3]. CC BY 4.0.*

In summary, our findings validate that nanodrug-induced cytotoxicity is not mediated by one singular pathway, but represents the outcome of a multidimensional network of cellular stress responses. Mitochondrial defects, lysosomal instability, ROS generation, inflammation, and membrane dynamics all contribute to nanodrug formulation cytotoxicity profiles. Through identification of such common molecular properties and application of AI-facilitated literature mining to systematically map risk profiles, this study establishes the foundations for rational design of safer, more effective nanomedicines. The proffered predictive metrics (the *Toxicity Index Scoring System* and the *Cytotoxicity Checklist*) present appealing methodologies for preclinical risk assessment and informed nanoparticle design in all therapeutic and diagnostic applications.

# 7. Declaration

I hereby declare that I have written the present dissertation entitled "*Nanoparticle-Cell Interactions: Mechanisms of Uptake, Lysosomal Processing, and Implications for Nanomedicine Safety and Therapeutics*" independently and without the use of any aids other than those explicitly referenced. All sources from which text has been quoted directly or paraphrased have been clearly cited in accordance with academic standards.

To enhance the depth and efficiency of the systematic analysis and literature review, I utilized Elicit AI (https://elicit.com, accessed on 5 May 2025), an artificial intelligence-based research assistant built on the Semantic Scholar corpus (https://www.semanticscholar.org/, accessed on 5 May 2025), which comprises over 126 million scholarly publications. In the course of preparing this dissertation, I also employed ChatGPT-5 (OpenAI) to support grammar refinement, academic style adjustments, English translation, and consistency checks.

All content generated or edited with the assistance of AI tools was critically reviewed, verified, and revised by me. I take full and sole responsibility for the scientific content, the interpretation of the data, and the overall integrity of the work presented herein.

Furthermore, I affirm that this research was conducted in accordance with the principles of good scientific practice, as outlined in the European Code of Conduct for Research Integrity published by ALLEA (All European Academies).

# 8. Acknowledgements

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# 10. List of Publications that Form the Basis of the Dissertation

(IF of the year in which article was published; citation from Web of Science without self-citations, 22.9.2025)

10.1. **Lunov, O.**; Syrovets, T.; Buechele, B.; Jiang, X.; Roecker, C.; Tron, K.; Nienhaus, G.U.; Walther, P.; Mailaender, V.; Landfester, K.; Simmet, T. The effect of carboxydextran-coated superparamagnetic iron oxide nanoparticles on c-Jun N-terminal kinase-mediated apoptosis in human macrophages. *Biomaterials* **2010**, *31*, 5063-5071. (IF = 7.883, citations = 139)

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