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A framework for designing delivery systems

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The delivery of medical agents to a specific diseased tissue or cell is critical for diagnosing and treating patients. Nanomaterials are promising vehicles to transport agents that include drugs, contrast agents, immunotherapies and gene editors. They can be engineered to have different physical and chemical properties that influence their interactions with their biological environments and delivery destinations. In this Review Article, we discuss nanoparticle delivery systems and how the biology of disease should inform their design. We propose developing a framework for building optimal delivery systems that uses nanoparticle–biological interaction data and computational analyses to guide future nanomaterial designs and delivery strategies.

Delivery is one of the most important research topics in the 21st century. Delivery is defined as the ability to bring an agent from outside the body (or biological system) to a specific targeted site in the body (or biological system). There have been significant advances in the development of new imaging agents, therapeutics and biological tools such as genome editors and nanomachines. These emerging technologies are exciting as they offer the ability to treat diseases with cellular and molecular precision. They are typically incorporated into nanoparticles, bacteria, viruses and other vehicles that protect them from degradation and enable them to be delivered to the biological target. However, the majority of the nanoparticles generally accumulate in off-target tissues. Delivery can vary depending on the size, shape, surface chemistry, stiffness and chemical composition of the delivery vehicles, but the optimal design to reach a specific biological target is unclear. The current design paradigm is mainly from a physical (that is, chemical and material properties) rather than a biological perspective. Administered nanoparticles interact with different tissues and organs that filter them out and prevent their delivery to the target site. The biological systems that a nanoparticle interacts with along its journey from the site of administration to the target disease site form barriers that control the delivery process. Accumulation in off-target locations causes harmful side effects and reduces the quantity of the drug at the disease site and thus the efficacy of the formulation. Here, we explore the role of biology and its barriers in guiding nanoparticle design for delivering medical agents to the target site.

The nanoparticle journey

Nanoparticle delivery systems interact with different molecules, cells, tissues and organs as they are transported through the body (Fig. 1). The relationship between engineered nanomaterials and a biological system is referred to as nanoparticle–biological (nano–bio) interactions. These interactions dictate what happens to the nanomaterial inside the body. When nanoparticles are administered, proteins instantly adsorb to the nanoparticle's surface and form a protein corona. The protein corona profile, composition and assembly depend on the biological molecules and fluids (such as blood, cerebrospinal fluid and saliva) the nanoparticle first interacts with and the nanoparticle's physical and chemical properties such as size, shape and surface charge[1,2]. This corona forms a new interface between the nanoparticles and cells or tissues, and influences nanoparticle uptake, biodistribution and immune response[2–5]. The protein corona may alter the nanoparticle's in vivo trajectory. This protein corona can also mask the targeting effect of engineered ligands on the nanoparticle surface[6,7]. Salvati et al. found that cell-specific targeting with transferrin conjugated nanoparticles decreased when the nanoparticles were coated in serum proteins compared to uncoated nanoparticles[7].

The majority of nanoparticles circulating in the blood are typically removed by the liver and spleen of the reticuloendothelial system (RES). The function of these organs is to filter blood and remove biological debris and foreign particulates from circulation. These organs form a significant barrier to intravenously administered nanoparticles including quantum dots[8], micelles[9], gold nanoparticles[10] and liposomes[11,12]. The liver can sequester the majority of intravenously administered nanoparticles and retain non-degradable nanoparticles for months after administration[13]. The primary source of nanoparticle sequestration are Kupffer cells. These phagocytic immune cells line the inside of liver sinusoids and capture nanoparticles as they pass by in circulation[14]. Tsoi et al. demonstrated that sinusoids slow blood velocity when compared to arteries and veins, increasing the probability of nanoparticle interaction and uptake by Kupffer cells[14]. This can make the RES system a suitable target for intravenously administered nanomedicines[15], as they will largely accumulate there. However, avoidance of the RES is essential for improving the delivery efficiency of intravenously administered nanoparticles to targets outside of the RES[16,17]. Other organs will also remove nanoparticles depending on their physical and chemical properties such as their size. For example, nanoparticles smaller than 6 nm can be renally excreted by the kidneys[18] and intradermally administered nanoparticles can be sequestered by dendritic cells[19]. These are only a few examples of the different cells and tissues that remove nanoparticles from circulation and prevent them from reaching the biological target site.

Nanoparticles that reach the target organ must exit the vasculature to reach target cells within the tissue. Nanoparticle transport through the blood vessel is dependent on the vessel physiology. For example, the vessels of the liver sinusoid are fenestrated, so nanoparticles smaller than the fenestrae (approximately <100 nm)

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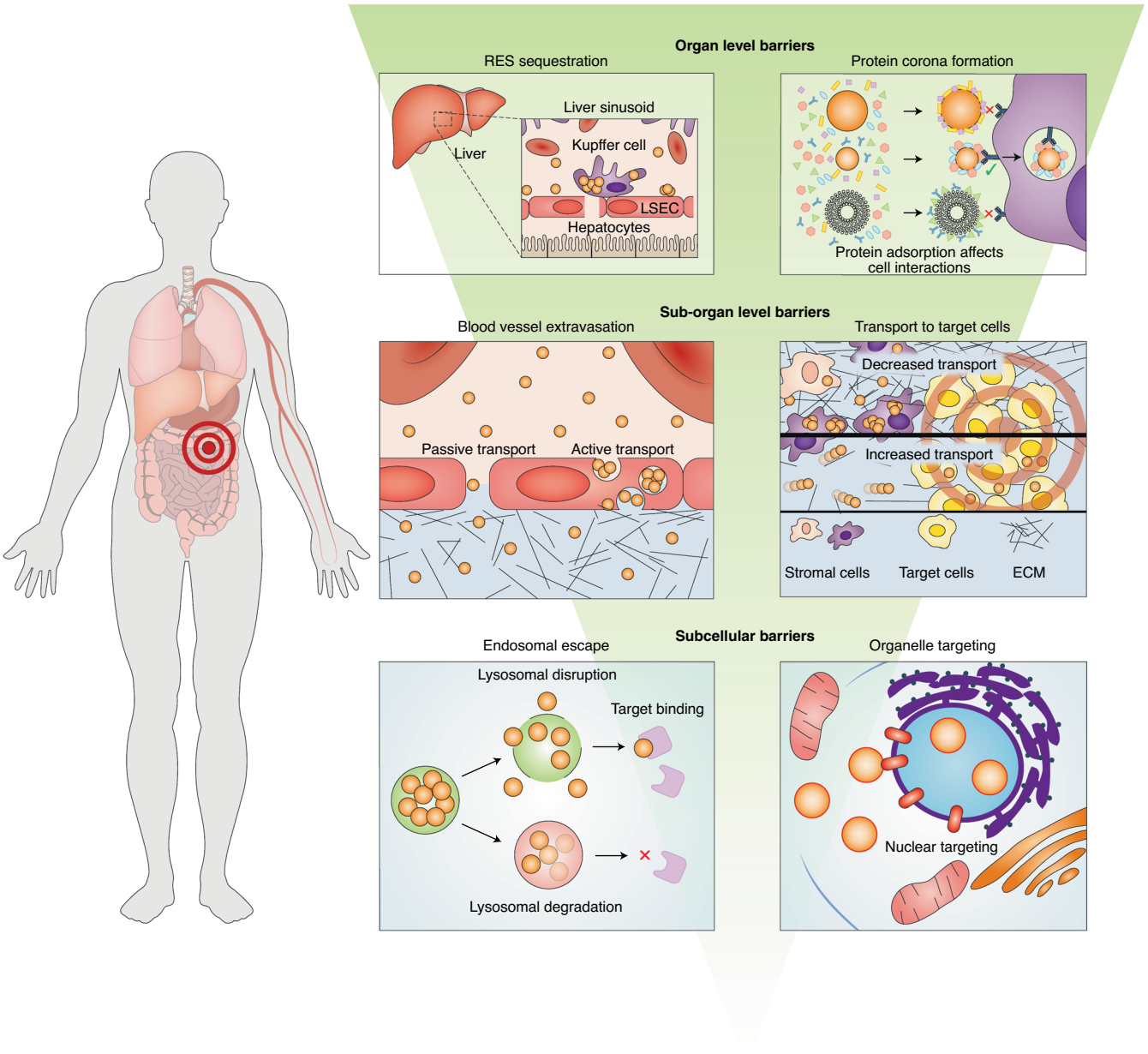


Fig. 1 | Biological levels of nanoparticle barriers. The number of delivery barriers increases with a deeper delivery target. Organs are typically the easiest to deliver nanomaterials to, while subcellular structures are the most difficult because the nanoparticles have more barriers to travel through to get to the final destination. RES, reticuloendothelial system; LSEC, liver sinusoidal endothelial cell; ECM, extracellular matrix.

can diffuse through to access the space of Disse[20]. The vessels in the glomeruli of the kidney also have fenestrae. The effective cut-off size is smaller (<6 nm)[18,21,22] due to the structure and composition of the underlying glomerular basement membrane[23–25]. Nanoparticle size[21,22], surface charge[26,27] and shape[28,29] can all affect nanoparticle clearance by the kidneys. The vessels in the brain are tightly regulated by a blood–brain barrier that prevents delivery of nanoparticles carrying drugs or imaging agents into the brain[30]. The vessels of solid tumours use a combination of both active and passive transport mechanisms to transport nanoparticles[31]. Vessel physiology varies across different endothelial linings. The physicochemical properties of the nanoparticle (such as size[20], charge[27] and surface chemistry[32]) should be designed to extravasate through blood vessels at the target tissue.

Nanoparticles inside the target tissue must travel through the tissue stroma to reach the target cells. The tissue stroma has extracellular matrix (ECM) and connective tissue cells, such as fibroblasts, pericytes and tissue-specific support cells. Extracellular matrix proteins can trap nanoparticles before they reach their intended target. The composition of the ECM varies between tissues and can be drastically altered in diseases such as liver fibrosis and cancer[33–35]. Some ECM components such as collagen, fibrinogen and hyaluronic acid can sterically hinder nanoparticle diffusion[36,37]. Off-target cells present within the stroma can sequester nanoparticles before they reach their intended target cell type. For example, tumour-associated macrophages can sequester nanoparticles that reach the tumour, preventing them from delivering their cargo to cancer cells[38–43]. Nanoparticles must navigate through the tissue stroma and avoid sequestration or degradation in the ECM or by off-target cells to reach their target.

Nanoparticles may need to enter the target cell for its cargo to elicit a therapeutic effect. Cellular uptake can occur through various

mechanisms such as membrane fusion[44], caveolin-mediated endocytosis[45], clathrin-mediated endocytosis[46], macropinocytosis[47] or phagocytosis[48]. The target cell phenotype and nanomaterial chemical composition determine how nanoparticles are taken up and processed. The surface receptor identities, expression levels and recycling kinetics affect the uptake rate and pathways accessible to the nanoparticles. This affects the optimal uptake route for a given cell type[49,50]. Santos et al. found that 132N1 cells mainly used clathrin-mediated endocytosis while A549 cells mainly used the caveolin pathway[50]. The physicochemical characteristics of the nanomaterial such as size, shape and surface chemistry also influence the uptake route. Meng et al. demonstrated that HeLa and A549 cancer cells took up ~40 fold more silica nanoparticles via macropinocytosis when the aspect ratio was 2.1–2.5 in comparison to other aspect ratios[47]. These examples show that there are a variety of cell type specific mechanisms that dictate nanoparticle–cell interactions and uptake.

Nanoparticles inside the cell may need to escape the endosome to reach the final subcellular target location, such as the cytoplasm. Strategies include the use of positively charged lipids to disrupt endosome bilayer stability[51] or pH sensitive polymers to modulate proton transport in endosomes[52,53]. In one application of this strategy, Hu et al. developed a pH-responsive polymer nanoparticle for cytosolic drug delivery. This nanoparticle responded to acidic lysosomes by increasing in diameter from 200 to 500 nm when the pH dropped from 7.4 to 4.9 to disrupt the membranes of acidic lysosomes[54]. This translated to a ~16-fold increase in cytosolic localization of the delivered cargo in dendritic cells in vitro compared to a non-pH-responsive design[54]. Some drugs may need to access organelles within the cell. Pan et al. demonstrated improved nuclear delivery of silica nanoparticles to HeLa cancer cells by conjugating the HIV-TAT nuclear localization peptide to the nanoparticle surface[55]. This nuclear localization sequence binds the α and β importin receptors (karyopherin) for active transport into the nucleus[56]. Methods allowing endosomal escape and using specific organelle localization tags should be considered for nanoparticles targeting subcellular locations.

Each level of nano–bio interactions has barriers that prevent nanoparticles from being delivered to the target site. Nanoparticles can be sequestered or degraded at every barrier, reducing the number of nanoparticles on the delivery journey to the target. Understanding interactions between nanoparticles and the biology at each level will help to design efficient nanocarriers optimized for the biology of the delivery pathway.

Barriers remove nanoparticles from the journey

Delivery efficiency can be defined as the percentage of administered nanoparticles delivered to the intended biological target. It depends on the number of biological barriers and how the nanoparticles interact with them (Fig. 2a,c). Using tumour targeting as an example, the amount of nanoparticles decreases as they move through the barriers (Fig. 2b). Conceptually, this is exemplified by the reduction in accumulation as they transport from the whole tumour to the cancer cell nucleus. Dai et al. showed that 0.7% of gold nanoparticles are delivered to the solid tumour, but only 0.0014% are delivered to the tumour cells in mouse models[38]. This is due to the biological barriers (for example, extracellular matrix and tumour associated macrophages) that a nanoparticle has to overcome to go from the tumour vessel to the final target cell.

The number of barriers can affect delivery efficiency. One approach to reduce the number of barriers is to change the administration route to directly bypass certain barriers. Many nanomedicines use intravenous delivery, which is suitable for targeting haematological, vascular or systemically disseminated diseases since there is direct access to these sites via the blood circulation. Oral administration allows access to the gastrointestinal tract, intraocular administration to the eye, inhalation to the lungs and intradermal to the skin and lymphatic system. Garbuzenko et al. showed that inhalation improved both the delivery to the lungs and the therapeutic efficacy compared to intravenous administration[57]. Intravenously administered nanoparticles must pass the barriers of the RES system (liver and spleen), and face degradation in circulation before reaching the lung. The inhaled nanoparticles have more direct access to the lungs and face fewer delivery barriers.

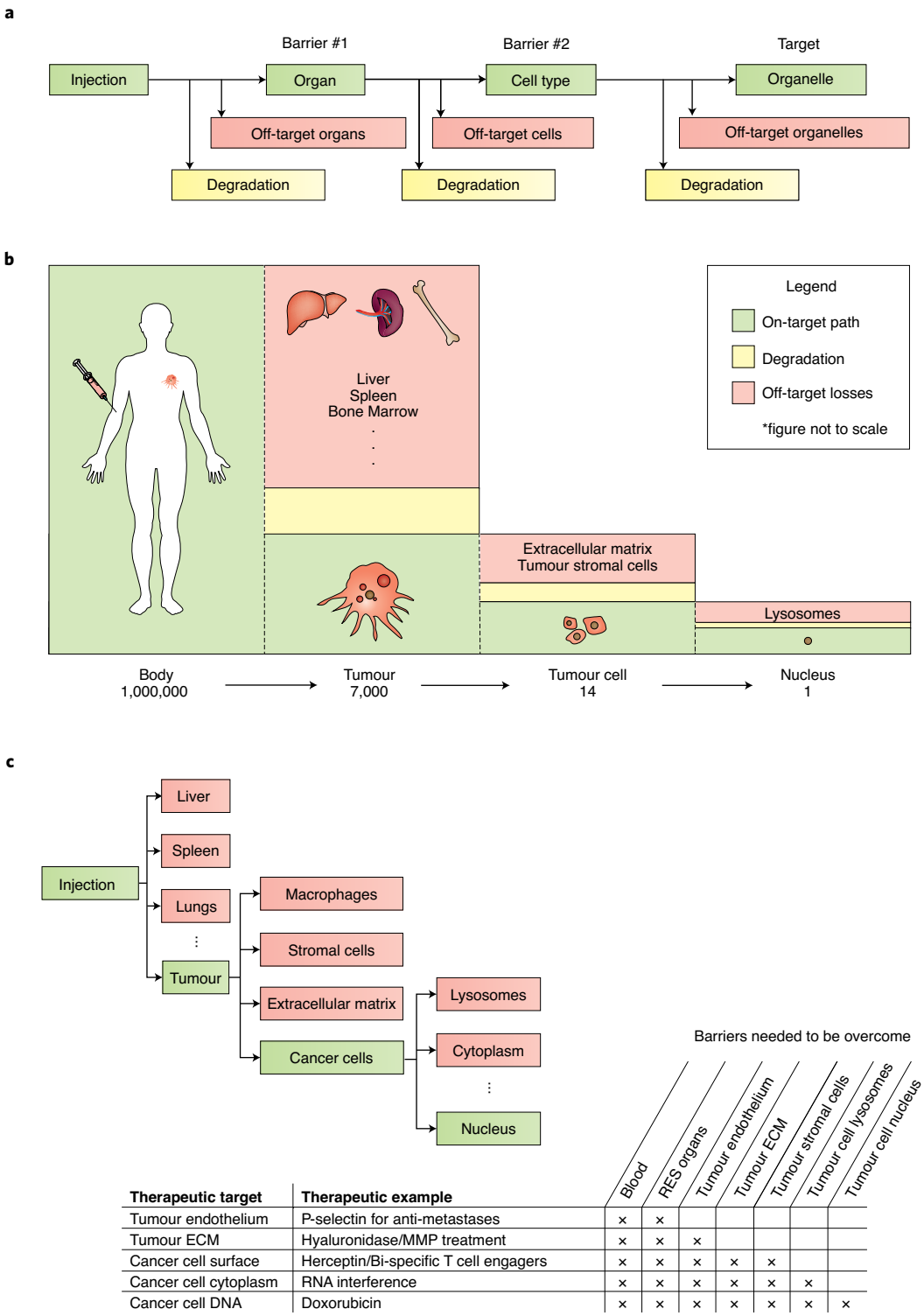
Another approach is to change the biological target. If the disease of interest has multiple druggable targets, the most direct delivery pathway may be selected in favour of higher delivery efficiency. In the example of treating a solid tumour in Fig. 2c, targeting the tumour endothelium has fewer barriers to overcome compared to targeting the nuclei of tumour cells. This concept is used by clinically approved nanomedicines. They target pathologies located in tissues where nanoparticles preferentially accumulate such as the skin[58] and the RES system[59]. Examples include: Doxil (liposomal doxorubicin) for the treatment of AIDS-related Kaposi's sarcoma skin tumours[60], and Feraheme (carbohydrate-coated iron oxide nanoparticle) for iron deficiency anaemia treatment in the liver and spleen[61].

Identifying the ideal nanoparticle design

Identifying the optimal nanoparticle physicochemical properties for delivery to a specific target is challenging. What is the best size, shape or surface chemistry for targeting disease x, y, or z? The ideal nanoparticle design is one that avoids off-target interactions and favours on-target interactions.

Collecting nano–bio interaction data. There have been a significant number of studies that aim to elucidate the relationship between the physicochemical properties of engineered nanomaterials and their interaction with biological systems in vitro and in vivo. However, it is a complex set of interactions that are unlikely to be defined by a single parameter. (1) Data from in vitro studies can be used to understand cellular or subcellular level nano–bio interactions. This is exemplified by in vitro studies investigating nanoparticle uptake by cells where essentially every property of the nanoparticle has an impact on cell uptake including size[62,63], shape[64–66], ligand density[67,68], material composition[69] and surface chemistry[70,71]. Bai et al. showed that palladium and gold nanoparticles were taken up more than platinum nanoparticles[69]. Wang et al. showed the ligand valency of the nanoparticle surface impacts their cellular interactions with SK-BR-3 and MCF-7 breast cancer cell lines[72]. (2) Data from in vivo studies are critical to delineate the role of multiple organs or systems in the delivery process. Nanoparticle libraries can be created and administered into animals to understand how different nanoparticle properties affect certain biological outcomes. Nanoparticle elimination from the body is one example where nanoparticle libraries have been used. Choi et al. established the 6 nm cut-off size for renal elimination using a library of different sized quantum dots[18]. Poon et al. used a library of gold nanoparticles larger than 6 nm to show that nanoparticles in this size range are eliminated through the hepatobiliary pathway in the faeces or retained in the liver long-term[20]. Collection of nano–bio interaction data at both the in vitro and in vivo level is required to determine the optimal design.

Nanoparticle interactions at the disease site are also important to understand. The physiology of the tissue at the disease site will be different than in the healthy tissue and can affect the nanoparticle delivery to the target cells. This has been studied in solid tumours where nanoparticle penetration and distribution is affected by collagen density[36], blood vessel density[73,74], blood vessel perfusion[75] and immune cell composition[39,42]. Sykes et al. measured the collagen content of solid tumours and then modelled nanoparticle diffusion through collagen gels at different collagen densities[36]. They found that larger nanoparticles (>60 nm) had reduced diffusion through higher collagen densities (>4 mg ml^{-1}) compared to smaller sizes and lower collagen densities. Ekdawi et al. measured vascular properties of solid tumours from mice injected intravenously with



Therapeutic target	Therapeutic example	Blood	RES organs	Tumour endothelium	Tumour ECM	Tumour stromal cells	Tumour cell lysosomes	Tumour cell nucleus
Tumour endothelium	P-selectin for anti-metastases	×	×					
Tumour ECM	Hyaluronidase/MMP treatment	×	×	×				
Cancer cell surface	Herceptin/Bi-specific T cell engagers	×	×	×	×	×		
Cancer cell cytoplasm	RNA interference	×	×	×	×	×	×	
Cancer cell DNA	Doxorubicin	×	×	×	×	×	×	×

Fig. 2 | A systematic view of nanoparticle delivery barriers. a, Schematic proposing a barrier framework of the nanoparticle delivery process. The administered dose of nanoparticles is removed by successive barriers in the body until only a small percentage is delivered to the intended target; **b**, An example of using the barrier framework to model nanoparticle drug delivery to the nucleus of a solid tumour. After the nanoparticles are administered intravenously, they have to transport through the bloodstream to get to the final target site. Many of these nanoparticles are taken up by the liver, spleen and other reticuloendothelial organs. Once they enter the solid tumour, they have to cross the blood vessel, extracellular matrix and other non-tumour cells before they reach the tumour cells. Then they would have to cross the cell membrane, vesicles and other subcellular structures before reaching the target in the nucleus. To illustrate how challenging this is, we show that one out of one million nanoparticles may reach the nucleus with the successive loss of nanoparticles along the delivery pathway. **c**, A detailed description of the different barriers a nanoparticle has to overcome to reach different therapeutic target locations for cancer therapy. MMP, matrix metalloproteinase.

fluorescent liposomes[73]. They found that the liposomes accumulated in areas of the tumour with high vascular density, such as the periphery of the tumour. Stirland et al. investigated the role of vascular perfusion on local nanoparticle accumulation within tumours by injecting two uniquely fluorescent nanoparticles either sequentially or together and analysing histology sections of the tumour[75]. They found that co-injected nanoparticles co-localized in the same parts of the tumour. When the two formulations were injected at different times, they accumulated in different areas of the tumour because the local blood vessel perfusion was dynamic. Cuccarese et al. investigated the impact of immune cell populations on nanoparticle accumulation by injecting lung tumour-bearing mice with fluorescent nanoparticles and then imaging the whole lungs for macrophage and nanoparticle distribution[42]. They found that the amount of nanoparticles in a tumour correlated with the number of macrophages in that tumour. Furthermore, identifying the ideal nanoparticle formulation is complicated by pathophysiological changes in disease states. The presence of disease can alter the in vivo nano–bio interactions and change the nanoparticle's blood clearance properties. Kai et al. determined that nanoparticle clearance from the blood is faster in tumour-bearing mice than healthy mice[76]. Similarly, Wu et al. showed in human patients that liposomal drugs eliminated 1.5-fold more quickly in patients with liver tumours compared to patients without liver tumours[77]. Collecting data on nano–bio interactions is the key step in determining the optimal design. The abundance of data will likely require computational analysis to identify how the complex relationships between the nanomaterial and biology allow for the identification of the optimal design.

Computational techniques to process nano–bio interaction data. Identifying the best nanoparticle design can be aided by experimentally examining how molecules, cells and tissues interact with nanoparticles of different designs. Many of the examples in the preceding sections were focused on understanding how a single parameter contributes to a single biological outcome. However, within the body there are many confounding and complex interactions between the nanomaterial and molecules, cells, and tissues that are not well understood. As the number of nanoparticle designs being tested increases and the amount of biological data collected about the nano–bio interactions increases, establishing complex relationships between these variables becomes possible. Computational techniques may be used to define the relationship between the nanomaterial properties and their biological interactions. Figure 3 shows a general, high-level overview of this framework. This is an emerging area of research and the aim of this section is to highlight examples of computational approaches for understanding nano–bio interactions.

Linear regression models. Linear regression models can be used to estimate the relationship between a dependent variable and one or more independent variables. The advantage of this method is that it is simple to implement and evaluate. The main disadvantage is that it does not accurately model non-linear relationships. Walkey et al. developed a partial least-squares regression model to predict cell association based on the protein corona of a panel of nanoparticle formulations[1]. A library of 105 different nanoparticle formulations (different sizes, materials and surface chemistries) were incubated with serum, and the protein corona was measured using liquid chromatography tandem mass spectrometry (LC-MS/MS). This data was used to generate a multivariable model that could predict cell association with 84% higher accuracy than single variable models using a single protein. This technique is typically used to establish the interaction of nanoparticles with simple biological systems such as cells in vitro.

Decision matrices. A decision matrix is a flow chart or table that can be used to identify key nanomaterial design parameters. Sykes et al. created a decision matrix to identify the optimal nanoparticle size for tumour accumulation for imaging (best contrast) or treatment (high retention and even distribution) applications based on tumour accumulation, fluorescence intensity, uptake rates, penetration capacity and theoretical loading capacity data that were ranked in importance for therapeutic and diagnostic applications. Each nanoparticle size was scored for its usefulness in imaging or treating tumours of different sizes[36]. Poon et al. created a decision flowchart to identify nanoparticle elimination pathways based on size and chemical composition[20]. This can be used to decide which nanoparticle design to use to access different elimination pathways. The advantage of decision matrices is that they are simple to implement and understand. The disadvantage is that they guide decisions for only a few simple design parameters.

Machine learning. To develop predictive models with limited knowledge of the relationships between the large number of variables, researchers have begun exploring machine learning methods (such as support vector machines, neural networks and random forests). Machine learning models are statistics-based computer algorithms that learn to perform a task without explicit instructions. When the relationship between a set of input variables (for example, nanoparticle characteristics) and a set of output variables (for example, tumour delivery) are unknown, machine learning can optimize a set of mathematical rules (that is, a model) to predict this relationship. Machine learning has been explored for applications such as to predict cell binding or accumulation in animals from the protein corona on nanoparticles[78–80], predict delivery to metastatic tumours[74], predict nanoparticle toxicity[81,82] and analyse chemical reagents for tuning nanoparticle physicochemical properties[83,84]. This usually requires hundreds to thousands of examples of accurately annotated data to train and validate the algorithm.

Support vector machines. Support vector machines are a method of supervised learning that can be used for classification or regression. Support vector machines are useful in situations with limited datasets (a few hundred data points) and when the prediction needs to tolerate noise in the dataset. Support vector machine models have been created to predict nanoparticle delivery to metastatic tumours based on tumour three-dimensional (3D) morphology[74] and to predict cell binding to different nanoparticle formulations from their protein coronas[79]. Kingston et al. built a model to predict nanoparticle delivery to metastatic tumours by training a support vector machine model from imaging data. Light sheet microscopy was used to measure tumour morphology (that is, volume, surface area, sphericity, number of cells, cellular density, cell distance to blood vessels) and nanoparticle delivery (that is, number of nanoparticle positive cells, mean nanoparticle intensity) of over 1,300 individual metastatic tumours. The support vector machine model was trained to predict the number of nanoparticle positive cells (output) from the morphological data about the tumour (inputs). The model was able to predict the number of nanoparticle positive cells with a Pearson correlation (r) of 0.94 and root mean squared error (RMSE) of 27 cells. This proof-of-concept study demonstrates how disease physiology can be used to predict nanoparticle delivery.

Neural networks. Neural networks work by relating input data to output results using linear and non-linear transformations. The relationships are strengthened by large datasets iterating through multiple hidden layers of transformations. Each iteration adjusts the transformations, which improves prediction accuracy. The ability to adjust the number, connectivity and data transformations throughout the layers of the network makes these methods capable of modelling complex relationships between the dependent and independent variable(s). Lazarovits et al. used neural networks to generate a computational model to predict organ accumulation

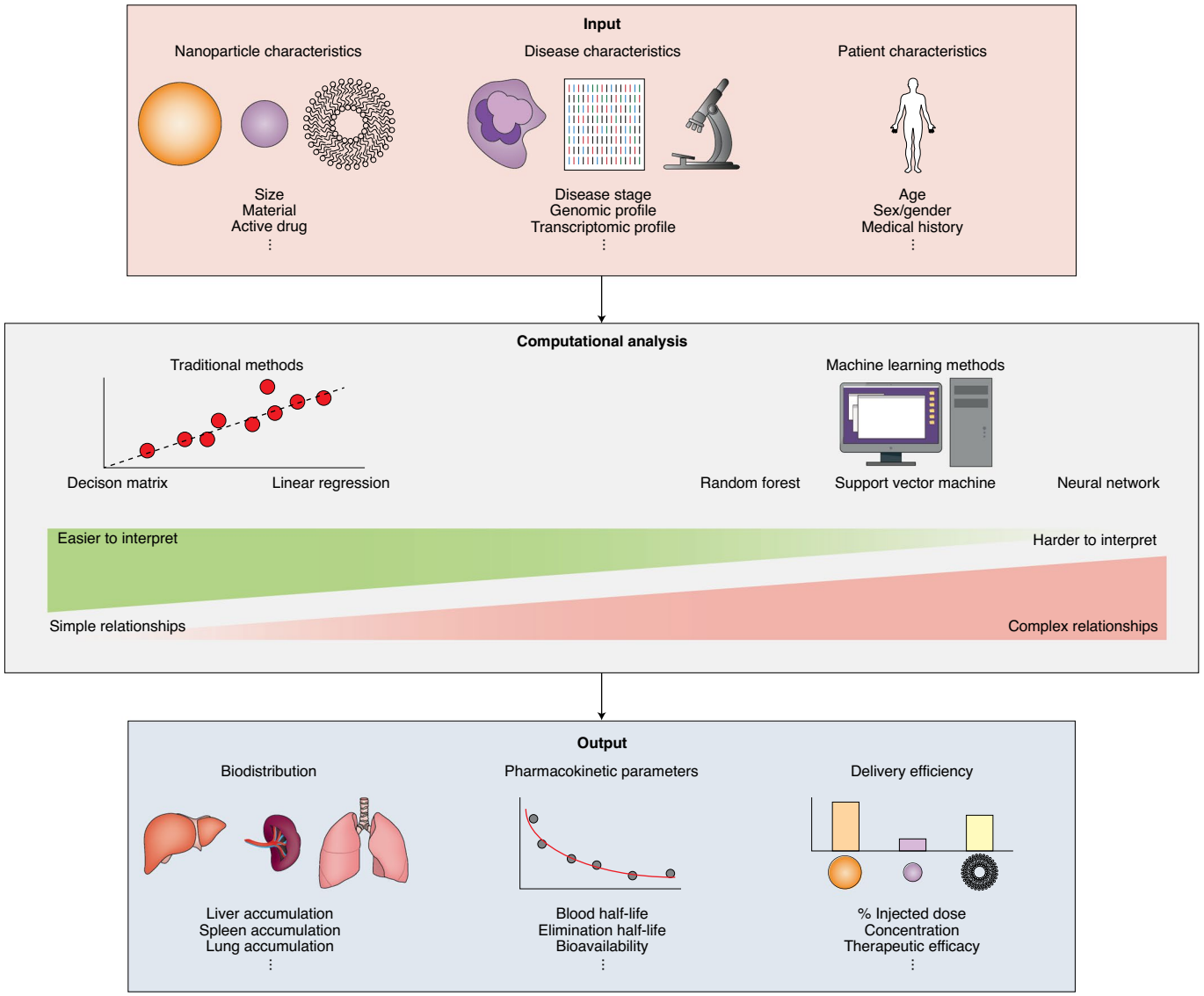


Fig. 3 | A computational framework for analysing nanoparticle–biological interaction datasets. Computational tools for understanding or predicting nanoparticle–biological interactions are useful for analysing large datasets containing information about libraries of different nanoparticles and their biological interactions. There are nanoparticle properties (size, shape, surface chemistry, material, surface charge) and disease or patient specific properties (disease stage, location in the body, phenotype, genomic profile, age) that impact the ability of a given formulation to reach the target disease site. Statistics-based, computational approaches provide tools that can be used to correlate multiple input parameters to the desired output parameters such as the biodistribution, pharmacokinetic profile or delivery efficiency of a given nanomaterial formulation to a specific delivery target.

and blood half-life using protein corona compositions from a panel of nanoparticle formulations[78]. Different gold nanoparticle sizes were injected into rats. Their protein coronas at different time points were used as input data. The output data was the amount of gold in the liver, spleen and blood. The neural network was trained to predict the amount of gold nanoparticles in the liver, spleen and blood based on the protein corona composition. When validated using two gold nanoparticle formulations of unknown composition the model was able to predict the half-life, spleen accumulation and liver accumulation based on the protein corona composition with 77–94% accuracy. This study demonstrated a proof-of-concept that the protein corona composition can enable prediction of nanoparticle delivery in animals.

Random forests. Random forests are an ensemble machine learning method for creating classification or regression models. Ban et al. used a random forest algorithm to predict the protein corona of nanoparticles given their physicochemical properties (size, shape, material, surface chemistry, surface charge)[80]. Data from the literature was collected from 652 different nanoparticles covering 40 different nanoparticle materials and over 50 types of surface modifications. Using 10-fold cross validation the random forest algorithm was able to predict the relative protein abundance of 178 different proteins with most R-squared values >0.7 and root mean squared errors below 5%. In the future, the prediction of the protein corona from the physicochemical properties of the nanomaterial could offer a way to screen nanoparticle interactions computationally.

Challenges of using computational approaches. A number of challenges exist for applying computational approaches to analyse nano–bio interactions. The first is the need for large datasets (hundreds to thousands of data points) of high-quality data. Measuring cellular uptake, or organ delivery across many different types of nanoparticles, in many different cell types or animals is costly and

time-consuming. In some situations, the amount of data required to achieve statistically significant relationships may be prohibitively large. Secondly, an interdisciplinary team of experts with knowledge across different areas including computer science, materials science and biological science is needed to deploy these methods. Thirdly, the algorithms need to be robust and work across a large variety of nanoparticle formulations or biological applications. In the above examples the models are used to solve a single problem, such as liver accumulation or cell binding for a limited library of nanoparticle formulations. These computational methods can be aided by data from studies that identify new biomarkers, cellular receptors and new nanomaterial designs. In the future, we need robust algorithms to predict multiple outputs from a large array of nanoparticle formulations and biological inputs.

Guiding the delivery strategy

The current sequence for engineering nano-delivery systems for in vivo applications is: (1) design and synthesize the nanoparticle, (2) characterize in cells in culture, (3) inject them into animals and measure delivery efficiency. The end therapeutic outcomes suggest whether the design was successful or not. This approach is based on a trial-and-error strategy. A reverse approach to the current strategy should be considered to achieve targeted delivery. This reverse approach would involve: (a) analysing the biology of the target disease, (b) defining the amount of nanoparticles to be delivered to each organ, (c) inputting results into algorithms, and then repeating steps (1) to (3) in the current sequence. A map of the nano–bio interactions can provide a blueprint for the optimum design of nanoparticles for the desired delivery efficiency. This method would also determine whether an adjuvant approach is required. An adjuvant's function is to alter the biology either at the diseased site or along the delivery pathway in order to improve the delivery efficiency of the nanomaterial by reducing the number or strength of the delivery barriers. Figure 4 outlines a workflow for delivery optimization that utilizes patient-specific data and knowledge on nano–bio interactions to guide the design of the delivery strategy. Box 1 defines a set of equations to conceptualize and compare the impact of barriers on the delivery of nanoparticles and their drug cargo to the target site. These tools describe a forward-thinking, patient-specific approach to designing the optimal nanoparticle and delivery strategy for reaching a specific disease target.

Biology determines the nanoparticle design. The biology of the diseased target site and the barriers the nanoparticles encounter along the delivery pathway determine the nanoparticle delivery efficiency. A workflow for the future design of nanoparticle delivery vehicles starts with using imaging and biochemical methods to assess the diseased target and the delivery barriers prior to engineering the nanosystem. The physiology of the disease will vary between patients and disease stages.

The first step is to analyse the disease target. Computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) together with contrast agents can assess organ-level barriers, such as perfusion, for nanoparticle extravasation and retention and predict nanoparticle target organ delivery[41,43,85,86]. Tissue biopsies and 3D imaging can identify sub-organ level barriers, such as changes in immune cell populations, their spatial distribution within the tissue microarchitecture and the vascularization status of the target[42,74,87,88]. For tumour targeting, this would include imaging and analysis of vascular architecture, vessel permeability, extracellular matrix porosity, biomarker expression, cell distribution and cytokine profiles. These imaging and molecular tests will inform the design of nanoparticles through the computational algorithms.

The second step is to use computational algorithms to calculate a nanoparticle design with optimal size, shape and surface chemistry.

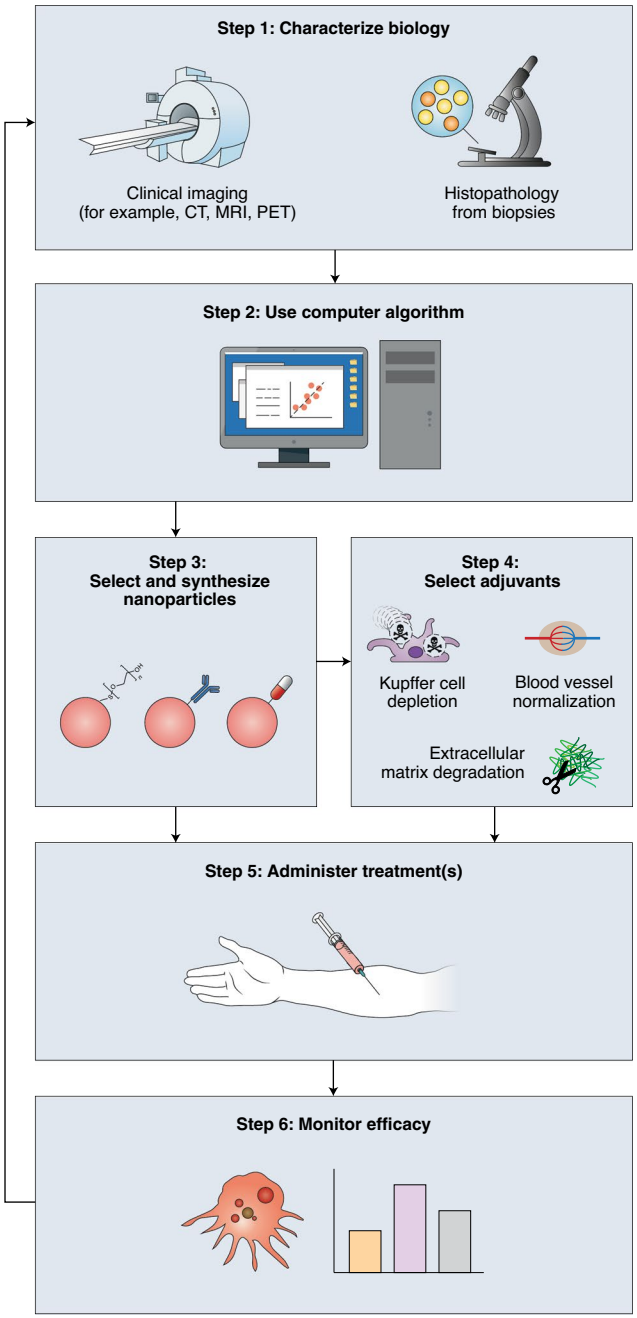


Fig. 4 | A rational strategy for designing and testing nanoformulations for delivery. The biology of the final target and the organs/cells that a nanoparticle interacts with will determine their delivery efficiency. We propose a new workflow for personalizing the design of the nanoparticles for a specific clinical application. This is a six-step process. (1) Analyse the pathophysiology of the diseased target. (2) Input the information and delivery goals into a computer algorithm. (3) An algorithm generates design specifications for the nanoparticle. Synthesize and characterize these nanoparticles. (4) Determine if an adjuvant is needed to increase delivery. (5) Inject into the patients either the nanoparticle alone, or with an adjuvant before, during or after injection of nanoparticles. (6) Monitor the clinical performance of the nanoparticle with or without adjuvant. During the treatment regimen, step 1 is repeated to measure changes in the disease, and off-target effects. Each round of treatment may require a different nanoparticle design as the pathophysiology and biological response may change. Kupffer cell image adapted with permission from ref. [20], American Chemical Society.

This design would be the most effective at overcoming the patient-specific barriers, determined in step 1 to achieve the desired delivery outcome. Changing the nanoparticle design can change both on-target and off-target accumulation (Box 1). The next steps are to synthesize this nanoparticle design, administer the nanoparticle therapy to the patient and to monitor the efficacy and changes in the pathophysiology of the target site to determine the next nanoparticle design iteration. This process shares similar philosophies to personalized medicine where the treatment is tailored to the patients' specific disease pathophysiology. Miller et al. showed that magnetic resonance imaging with an iron oxide nanoparticle (Feraheme)-based contrast agent could be used in tumour bearing mice to predict the delivery and therapeutic response of a subsequently administered therapeutic nanoparticle[41]. Ultimately, the biology informs the optimal design of nanoparticles to achieve maximum delivery.

Priming the biology for delivery. Even the optimal nanomaterial design may not ensure enough nanoparticles are delivered to the target site. One may need to add adjuvant strategies to improve the delivery efficiency. These strategies inhibit or block specific organs or cells from taking up nanoparticles en route to the target site, which would reduce the off-target accumulation (Box 1). This allows more nanoparticles to reach the final target site. Designing these strategies requires a thorough understanding of how specific biological barriers influence nanoparticle transport in a given delivery pathway. Below are three current examples from cancer nanomedicine:

The first method is to disable the RES from taking up intravenously administered nanoparticles. This can be done by depleting or inhibiting Kupffer cells in the liver that sequester nanoparticles. Tavares et al. showed that depletion of liver macrophages improved tumour accumulation by up to 150 times depending on the nanoparticle type and cancer model[89]. Other strategies used decoy nanoparticles to block RES uptake of subsequent nanoparticle injections and increased their circulation half-life[90–92]. Ouyang et al. recently showed that the administered dose of any nanoparticle design must exceed a general dose threshold to overwhelm the RES uptake rates and maximize delivery to tumours up to 12% of the injected dose[93]. In mice, this threshold was 1 trillion nanoparticles and this highlights the influence of dose on nanoparticle delivery strategies. By reducing uptake in RES organs, nanoparticles are able to circulate longer and increase their potential to accumulate at the target site.

A second method is to improve transport of nanoparticles out of blood vessels. Administering a vascular normalization agent (such as anti-VEGF or anti-VEGFR-2) can improve tumour blood vessel perfusion. This decreases tumour interstitial fluid pressure and increases blood supply[94–96]. Chauhan et al. showed that pre-administration of anti-VEGF-receptor-2 antibody DC101 improved the transvascular flux of 12 nm quantum dots by 2.7 to 3.1 times in mice with orthotopic mammary tumours[94]. Other strategies include using radiation therapy to make tumour blood vessels more permeable before administering nanoparticles[97,98].

A third method is to improve the diffusion of nanoparticles through extracellular matrix (ECM) to penetrate deeper into target tissues. Collagen and hyaluronic acid are major components of the extracellular matrix and are overexpressed in pathologies such as fibrosis and cancer[37,99]. Enzymes such as collagenase[100] or hyaluronidase[101] can be used to degrade these extracellular matrix proteins. Sparse ECM can also increase vessel permeability by opening blood vessels[102]. Murty et al. showed that attaching collagenase to their 30 nm gold nanoparticles improved tumour delivery by 1.4 times[103]. Eikenes et al. pre-treated human osteosarcoma tumour-bearing BALB/c nude mice intratumourally and intravenously with hyaluronidase to increase tumour uptake of liposomal doxorubicin by 2–8 times[104].

Continuous analysis of disease biology. A treatment regimen may require different nanoparticle designs over time. For example, round 1 of therapeutic treatment of patients with stage 4 breast cancer may require a nanoparticle that is spherical and 50 nm. After initial treatment leading to some tumour shrinkage, the optimal nanoparticle design for the next injection may be 15 nm. It is possible that as tumour volume and ECM density changes, altering the nanoparticle design may be necessary[36,105]. Changes in the diseased tissue may also cause other systemic changes to metabolism and elimination, which could alter the ideal nanoparticle design

Box 1 | Equations comparing the delivery efficiency of nanoparticles, drugs and drugs carried by nanoparticles

We describe nanocarriers containing releasable small molecule therapeutics as a model system. We present simple mathematical equations to describe the delivery efficiency of the nanoparticles alone, small molecule drugs alone, and the drug and nanoparticle together, being transported to the target site. The delivery efficiencies of both the nanoparticles and small molecule drugs are directly related to the amount that accumulates in the target or inversely related to the summation of their losses due to the barriers that sequester or eliminate them. The therapeutic effectiveness of a nanoparticle carrying a drug is dependent upon the total drug delivered by the nanoparticle. This depends on the amount of nanoparticles delivered to the biological target, the amount of drug remaining in the nanoparticles that are delivered and the amount of drug released at the target. $\#NP_{\text{total}}$ refers to the total number of nanoparticles administered. $\#NP_{\text{on-target}}$ refers to the number of nanoparticles that are taken up at the intended destination. $\#NP_{\text{off-target}}$ refers to the number of nanoparticles that are taken up by off-target sites. $\#NP_{\text{liver}}$ refers to the number of nanoparticles that are taken up by the liver. $\#NP_{\text{spleen}}$ refers to the number of nanoparticles that are taken up by the spleen. $Drug_{\text{remaining}}$/NP refers to the amount of drugs still bound per nanocarrier. Release refers to the proportion of drugs that can be released by the nanoparticles. Of note, these equations can be written in different forms.

From the nanoparticle perspective:

$$\text{Delivery efficiency}_{\text{NP}} = \frac{\#NP_{\text{on-target}}}{\#NP_{\text{total}}}$$

$$\#NP_{\text{total}} = \#NP_{\text{on-target}} + \#NP_{\text{off-target}}$$

$$\#NP_{\text{off-target}} = \sum_{i=1}^{n} \#NP_{\text{off-target},i} = \#NP_{\text{liver}} + \#\text{NP}_{\text{spleen}} + \cdots$$

From the drug perspective:

$$\text{Delivery efficiency}_{\text{drug}} = \frac{\text{Drug}_{\text{on-target}}}{\text{Drug}_{\text{total}}}$$

$$\text{Drug}_{\text{total}} = \text{Drug}_{\text{on-target}} + \text{Drug}_{\text{off-target}}$$

$$\text{Drug}_{\text{off-target}} = \sum_{i=1}^{n} \text{Drug}_{\text{off-target},i} = \text{Drug}_{\text{liver}} + \text{Drug}_{\text{spleen}} + \cdots$$

$$\text{Drug}_{\text{on-target}} = \#\text{NP}_{\text{on-target}} \times \frac{\text{Drug}_{\text{remaining}}}{\text{NP}}$$

$$\text{Drug}_{\text{released}} = \text{Drug}_{\text{on-target}} \times \text{Release}$$

$$\text{Effective Dose}_{\text{Drug}} = \text{Delivery efficiency}_{\text{NP}} \times \#NP_{\text{total}} \times \frac{\text{Drug}_{\text{remaining}}}{\text{NP}} \times \text{Release}$$

Table 1 | Critical questions for designing nanoparticle delivery systems

Question	Rationale for the question
(1) Where is the delivery target?	The specific biological target (organ or tissue, cell type and subcellular location) defines design of the nanoparticle strategy.
(2) What is the cargo or active agent that needs to be delivered to the target location?	This defines the chemistry for incorporating the agents into the nanoparticle for delivery.
(3) Where is the site of administration?	The location of administration and the delivery target location define the delivery pathway.
(4) What are the specific organs, tissues and cells encountered along the delivery pathway?	This defines the barriers that the nanoparticle will encounter.
(5) What are the interactions between the nanoparticle carrier and the body in each of these biological environments along the delivery pathway?	These interactions will determine if the formulation is degraded or sequestered before it can reach its intended target location.
(6) What strategies are available to overcome the barriers at each step in the delivery pathway?	This allows the development of specific strategies to overcome the barriers.
(7) How will any administered components leave the disease site and be excreted from the body?	This helps to define locations of toxicity and elimination routes.

and delivery strategy[106]. Dose can also be modified in response to toxicity in off-target sites during monitoring[93,107]. Information from every iteration further improves the prediction accuracy and suggests more efficacious and tolerable regimens[108].

Perspective and outlook. We provide our view of where we believe the field of delivery is headed. We have witnessed the development of methods to synthesize and characterize nanoparticles, and demonstrate their utility in targeting, imaging and treating disease. This has created a foundation with competencies that will advance this important field of research. However, the approach is a trial-and-error process to discover one optimal particle design at a time. There are now several groups that are designing high-throughput libraries of nanoparticles for delivery and screening against diseased targets in vitro and in vivo to identify an optimal formulation[109–113]. While this is an excellent approach, we would need to assess the relevance of the screening results between species (for example, mice versus humans).

The broader concept behind our proposed strategy will lead to a more rational approach for engineering nanoparticles as delivery vehicles of medical agents. The approach is based on the principle of identifying relationships between nanoparticle design and in vivo transport at the organ level, sub-organ level or subcellular level, and then organizing these relationships into a system that can be analysed by computational algorithms to guide the design of the delivery vehicle and the delivery strategy. The implementation of artificial intelligence approaches in the clinic is an emerging area of research supported by new policies put forth by regulatory agencies such as the US Food and Drug Administration (FDA)[114]. In particular, there has been success in the clinic with the first FDA-approved, adaptive, personalized approach for radiation therapy in oncology powered by artificial intelligence[115]. Patient-specific radiation dose and positioning were optimized based on multimodal tumour imaging throughout the treatment regimen. We envision this futuristic approach could be applicable for nanomaterial-based chemo-, radio- and immuno-therapies once sufficient high-quality datasets of nano–bio interactions are available.

These developments will evolve the field of delivery to look at the delivery problem not only from the engineering and chemistry perspective, but also from a biological perspective. It is important to change our viewpoint on delivery as the biological systems ultimately determine whether the nanoparticles are able to access the final target site. Table 1 outlines a series of critical questions to consider when trying to optimize delivery to a specific target. These questions are designed so that the nano–bio interactions along the entire delivery pathway are considered when designing a strategy to reach a target disease site. Importantly, these design considerations apply to all nanoparticle-based delivery systems, including delivering drugs for therapy or imaging contrast agents for diagnostics, since achieving a high delivery efficiency and specificity for a target site is crucial to their clinical purpose[116]. In the future, we imagine being able to select a disease site and use algorithms to guide nanoparticle designs. For example, a researcher develops a drug and aims to deliver at least 10% of an injected dose to a tumour, with a maximum acceptable off-target delivery of 20% to the liver. The researcher inputs this desired delivery profile into a computer algorithm, which then calculates the ideal nanoparticle strategy. While we are currently far from implementing this strategy, we foresee this future for the rational design of delivery vehicles to bring emerging medical agents to a specific disease site.

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Competing interests

The authors declare no competing interests.

Additional information

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