Published in final edited form as:

Adv Drug Deliv Rev. 2013 May ; 65(5): 607–621. doi:10.1016/j.addr.2012.05.012.

Nanoparticle mediated non-covalent drug delivery[☆]

Tennyson Doane and Clemens Burda^{*}

Department of Chemistry, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106, USA

Abstract

The use of nanoparticles (NPs) for enhanced drug delivery has been heavily explored during the last decade. Within the field, it has become increasingly apparent that the physical properties of the particles themselves dictate their efficacy, and the relevant non-covalent chemistry at the NP interface also influences how drugs are immobilized and delivered. In this review, we reflect on the physical chemistry of NP mediated drug delivery (and more specifically, non-covalent drug delivery) at the three main experimental stages of drug loading, NP–drug conjugate transport, and the resulting cellular drug delivery. Through a critical evaluation of advances in drug delivery within the last decade, an outlook for biomedical applications of nanoscale transport vectors will be presented.

Keywords

Nanomedicine; Drug delivery; Nanoparticles; Non-covalent

1. Introduction

The use of nanoparticles (NPs) for the delivery of drugs is advantageous due to high surface to volume ratios, [1] modifiable platforms, [2] and tunable size [3]. Nanomedicine has benefited greatly from the unique properties of NPs, with a variable explosion in experimental and theoretical understanding within the last decade. Although commercial products for nanomaterials have yet to be widespread in the medical field, the development of new delivery and diagnostic agents holds promise for the future treatment of difficult diseases such as cancer [4]. Despite the advances in synthesis, characterization, and understanding of in vivo function, a quantitative understanding of what forces dictate NP–drug conjugate stabilization, transport, and drug delivery can be difficult to assess. In the realm of non-covalent drug delivery, these considerations are critical for designing an effective therapeutic. Unlike their covalent counterparts, non-covalent drug delivery vectors can be much more difficult to control and predict in complex media despite recent successes in vivo [5,6]. In either case, understanding the fundamental properties which determine efficacy is vital for the advancement of nanomedicine.

It is worthwhile pointing out that nanomedicine has many parallels to the chemistry of biomacromolecules, and protein chemistry in particular. Early in the days of nanomedicine it had been recognized that nature operates on the nanoscale in the form of macromolecules such as proteins and DNA/RNA, allowing the rich functionality that enables life. There are many similarities between colloidal nanoparticles and nanoscale proteins, including their

[☆]This review is part of the *Advanced Drug Delivery Reviews* theme issue on “Inorganic nanoparticle platforms.”

© 2012 Elsevier B.V. All rights reserved.

^{*}Corresponding author: Tel.: +1 216 368 5918; fax: +1 216 368 3006. burda@case.edu (C. Burda).

size, functionality, complexity, and the ability of multimodal activity, as noted by Karshikoff [7]. Many of their interaction with their environment are mainly based on non-covalent forces including van der Waals, steric, hydrogen bonding, hydrophobic and electrostatic interactions [7]. The overall balance among all these forces together can lead to surprising, non-intuitive properties in proteins as well as in nanoparticulate structures. Much of this discussion has already been identified within the past century through studies of small molecule interactions with biomolecules. Thus the idea of nanoparticle mediated drug delivery (and more specifically, polymer coated NPs) is, in essence, a reflection of current science and engineering learning from nature [8]. Therefore, it is important to highlight some of the aspects that nanoparticle based drug delivery and protein–molecule interactions share, including cooperative forces (Section 2.1.1) solvent interactions (Sections 2.1.1 and 2.1.2), instability due to biological conditions (Sections 2.2.4 and 3.1.1), conformational flexibility (Section 3.2.2), structure–function relationships (Section 3.3.3), and thermodynamic factors (Section 4). Through an exploration of the non-covalent forces present in NP systems, comparisons to bio-macromolecule transport can reveal new insights into how to better design NP based drug delivery vectors.

In light of the similarities between biological transporters and NPs, this review will explore the three phases necessary for in vivo NP mediated drug delivery: drug loading/conjugate stabilization, conjugate transport, and finally drug uptake. Specifically, we will focus mainly on the physical properties of the NP which will dictate the so called “passive” interactions with both drugs and the complex environments that in vivo systems present [9]. Through a careful exploration of how the field has progressed within the last decade, an outline of what physical properties dictate NP efficacy can be established which can guide future rational design criteria and accelerate the transition of nanomedicine to the clinic.

2. Drug loading and conjugate stabilization

The first phase necessary for effective drug delivery utilizing NP vectors is a determination of the loading efficiency and whether the resulting conjugates are stable. Although these optimizations seem intuitive, the practical realities facing nanomedicine demand a rigorous understanding of the physical chemistry at play in the conjugate as a whole. Here, we first discuss common methodologies for drug loading and assess the effect of these methodologies on the resulting conjugate stabilization, drawing on relevant literature to determine which optimizations are most critical for effective NP–drug vector design.

2.1. Drug loading

The loading of drugs onto inorganic NPs has been extensively investigated during the last two decades due to their promise as both a robust scaffold and highly tunable cargo vector [10]. When combined with the possibilities for inherent optical tracking of particles in vivo, the use of NPs as drug vectors has expanded nanoscience into the so called “theranostic” regime, in which multi-modality allows for simultaneous imaging and therapeutic action [11]. While loading of drugs onto NPs can seem straightforward, understanding how these drugs incorporate and modify the physical particle properties can be challenging. Here we review the physical chemistry at play during drug loading, payload optimization, and subsequent morphological changes to the vector through a review of recent successes in the literature.

2.1.1. Methods for drug loading—The use of NPs for the delivery of drugs is motivated by poor drug solubility in aqueous environments, early recognition and clearance by the body, and reduction of unwanted side effects [12]. All drug loading schemes are either based on covalent or non-covalent drug approaches, where covalent methods involve chemically bonded tethers and non-covalent drug delivery incorporates all other means of drug delivery

including hydrophobic [13,5,14], electrostatic [15], hydrogen bonding [16], and steric immobilization [17,18]. Furthermore, these common approaches can be further divided into either surface mediated or encapsulation techniques (Fig. 1) [12]. Interestingly, despite these distinctions for NP mediated drug delivery, both covalent and non-covalent methodologies require optimal loading sites and minimal hindrance to diffusion from the NP vector upon reaching the target site. These similarities are both particle dependent, where the drug loading must be facilitated by a favorable environment near the NP. As non-covalent drug delivery is much more sensitive to physical forces within the local environment, we will focus on this approach specifically.

Both inorganic NP mediated encapsulation [17–19] and surface mediated [5,14] non-covalent drug loading are prevalent in the literature. Invariably the loading of drugs non-covalently is dependent on multiple physical forces within the NP–drug conjugate. For example, Cheng et al. proposed the incorporation of an amino functionalized silicon phthalocyanine on PEGylated Au NPs as a result of non-covalent bonding to the Au NP surface [5], based on largely hydrophobic interactions. Subsequent work found that a net negative charge due to anion adsorption on the Au NP surface coordinates with the protonated amine to further promote and stabilize drug loading on the NP [20]. Results from the work of Rotello also may further support that hydrophobic drug loading is supported by electrostatic interactions, with a dramatic increase in tamoxifen (which bears an amine functionality) absorption to Au NPs which does not linearly correlate with its partition coefficient (logP) [14]. Thus the loading of drugs onto NPs is the summation of all cooperating forces within the system, and optimization of drug delivery will require an understanding of which forces are dominant in NP–drug interactions.

2.1.2. Drug loading efficiency—In both, covalent and non-covalent drug loading methodologies, the efficiency of attaching drugs to the NP carrier is of pivotal importance for successful therapy. In general, a higher drug loading efficiency is desirable for in vivo applications; however, with improved drug development, these criteria may be relaxed for the treatment of a specific disease. Both accessible binding sites and drug diffusion are significant (though not exclusive) limiting factors for drug loading efficiency. For NP-mediated drug incorporation, the amount of available space for bonding and stabilization will be critical for optimizing drug payload, both of which are correlated to the size and local environment of the NP. For covalently bound drugs, the binding sites need to be accessible and free of possible hindrances such as high electrostatic repulsion, highly dense and rigid coatings with small pore size, etc. Similarly, non-covalently bound drugs should be able to diffuse to the surface during loading, while the target environment needs to be kinetically and thermodynamically accessible from the NP corona during delivery. During the stage of drug loading, both the properties of the NP and the local environment are highly dependent on sample handling (solvents, ionic strength, etc.) and the design of the NP (core material, coating ligand, etc.). An excellent example of physiochemical dependent drug loading is the incorporation of hydrophobic drugs on water soluble NPs (Fig. 2). Kim et al. found that the incorporation of dyes onto zwitterionic surface assembled monolayer (SAM) coated Au NPs could be achieved by solvent replacement (Fig. 2A) [14], in agreement with work by Cheng et al. utilizing PEGylated Au NPs and a hydrophobic photodynamic therapy drug [5]. Lucarini et al. have studied this behavior via monitoring electron spin [21], and have shown that this process is NP core diameter dependent, which they attributed to different packing on the Au NP surface [22]. The same general diffusion based loading strategy has also been demonstrated for mesoporous silica nanoparticles as well (Fig. 2B) [13]. Considerations for loading solvent and specific material properties have also been reported for both hydrogen bond stabilized drug loading [23,16] and electrostatic drug loading [24].

It is important to note that like all drug delivery mechanisms, determining the relative amount of free drug to bound drug is crucial for understanding loading efficiency. Unlike covalent systems, however, non-covalent systems involve dynamic processes based on their local environment. Purification of the conjugates, then, can pose different challenges in comparison to drug delivery relying on covalent bonds. In the case of hydrophobic drug delivery, this is often assumed to occur during re-suspension of the particles in an aqueous environment [5]. In the case of other forces (steric stabilization and electrostatics, for example), this is usually accomplished by studying changes in the drug itself (such as diminished or enhanced fluorescence, bond stretches, reactivity, etc.). For example, it is well known that gold nanoparticles can effectively quench the fluorescence of drug molecules, and this phenomenon has been utilized to determine not only relative amounts of bound drug through the comparison of absorption to fluorescence spectra, but also subsequent drug release [25]. Future work in nanomedicine must continue to develop better methodologies for assessing incorporated versus free drugs after loading on the NP vector.

2.1.3. Changes to NP structure?—Knowing that the physical NP properties dictate the efficiency and mechanism of drug loading, it is also important to highlight that drug loading alters the NP structure. The incorporation of a hydrophobic drug onto a NP modifies the local solvent environment, ligand behavior, and subsequent loading of more hydrophobic molecules, resulting in possible aggregation of the NPs [5]. Fig. 3 shows PEGylated Au NP–drug conjugates in solution as a function of drug:NP ratio. While the NP conjugates are stable at ratios of 30–50, ratios above this critical limit result in particle aggregation [5]. Thus although a maximum drug loading efficiency is desired, its effects on conjugate stability must always be considered.

In addition to stability, the change in physical properties of NPs may also affect its effectiveness for targeting a specific disease. Recently, Lee et al. reported a dramatic difference in tumor uptake between a loaded and unloaded glycol chitosan NP vector [26]. The authors concluded that changes to the physiochemical properties of the NP resulted in this behavior, and stressed that it is important to understand the transport of a NP, both with and without carrying a payload [26]. The inherent complexities of biological environments and processes further necessitate an understanding of *which* NP properties are essential for the delivery of a specific drug.

2.2. Conjugate stabilization

Successful loading of drugs onto NPs is often determined simply by whether the resulting conjugates are stable, and this is directly correlated to the NP conjugate solubility. However, it has become increasingly clear that assessing the biological interactions of the NP–drug conjugates in solution or in vivo is vitally important. For example, Cho et al. have recently reported that the sedimentation of NPs can dramatically over estimate the uptake of NPs in cells, questioning whether previous in vitro experiments are an accurate representation of how NPs behave in real systems [27]. Although a given NP–drug conjugate may be stable in a pure solvent under ideal conditions, tests for functionality and stability in the complex and variable environment of in vivo biological fluids demands an understanding of what forces keep NPs soluble. Here, we apply the considerations of traditional colloidal science to examine how the incorporation of drugs onto NPs modify their stability, and critically evaluate which aspects are the most relevant in biological media.

2.2.1. van der Waals attraction forces—The tendency of NPs to form larger super-structures is well known in colloidal science. The phenomenon of irreversible aggregation was first defined as coagulation, whereas reversible aggregation is termed flocculation [28]. The driving force for aggregation is the attractive van der Waals force between two

particles. The attractive potential between two spherical particles of radius R can be obtained through the Derjaguin approximation of van der Waals formula for flat surfaces defined as:

$$V_{vdW} = A_H / 6 \left[\frac{2a_1a_2}{R^2 - (a_1 + a_2)^2} + \frac{2a_1a_2}{R^2 - (a_1 - a_2)^2} + \ln R^2 - \left(\frac{a_1 + a_2}{R^2 - (a_1 - a_2)^2} \right) \right], \quad (1)$$

where R is the distance between the two particles, a_1 and a_2 are the radii of each interacting particle, and A_H is the Hamaker constant which takes into account the polarization of the specific atoms of the material and their respective dielectric constants [29]. This formula can be further simplified if each particle has the same radius to [29]:

$$V_{vdW} = A_H / 12 \left[\frac{1}{x(x+2)} + \frac{1}{(x+1)^2} + \ln \frac{x(x+2)}{(x+1)^2} \right], \quad (x = R/2a). \quad (2)$$

Readers interested in a deeper understanding of the derivation of Eq. (1) and the complexities associated with the attractive forces between particles are directed to works by Hunter and Russel et al. [28,30]. For our purposes, note that (A) as the Hamaker constant increases so too does the attraction potential between the two particles (Fig. 4A), and (B) as A_H increases the force becomes more significant (Fig. 4B). In addition, the Hamaker constant is dependent on the local medium between the two particles [31]. Thus a critical design aspect is the NP core material. When rationally designing a NP–drug delivery vector, careful assessment of the forces needed to stabilize the conjugate is important. When drugs are attached (either covalently or non-covalently) to the NP, there is a degree of perturbation to the colloidal system which may decrease the overall stability, requiring further means of stabilization (as seen in Fig. 3).

2.2.2. Charge based stabilization—The use of charge to stabilize NPs has a rich history in the literature, with the citrate capped Au NP as a classic example [32]. The well known Derjaguin, Landau, Verwey, Overbeek (DLVO) theory states that the total interaction of a colloidal particle can be obtained by taking into account the attractive van der Waals forces and all relevant counter-forces, including electrostatic repulsion [33]. The repulsive force can be approximated for particles with equal a by [28]:

$$V_R = 2\pi\epsilon\psi_0^2 \ln[1 + \exp(-\kappa R)] \quad (\kappa a > 10) \quad (3)$$

or

$$V_R = 2\pi\epsilon\psi_0^2 \exp(-\kappa R) \quad (\kappa a < 5) \quad (4)$$

where

$$\kappa = \left[\frac{2000FI}{\epsilon k_B T} \right]^{1/2} \quad (5)$$

with ψ_0 defined as the surface charge, ϵ the product of the dielectric permittivity of vacuum and relative permittivity of the solvent ($\epsilon_0\epsilon_r$), κ is defined as the Debye parameter (in nm⁻¹), F is Faraday's constant, I is the ionic strength, k_B is the Boltzmann constant, and T is the temperature [28]. Thus for “bare” particles in solution with significant surface charge, the electrostatic repulsion between two particles is the primary method for stabilization.

However, this repulsive force will be highly dependent on the ionic strength of the solution (Eq. (5)), which correlates with the degree to which surface charge is effectively screened from the bulk media (qualitatively expressed through the effective particle charge known as the ζ -potential, ζ). In addition, the higher temperature of in vivo environments must be taken into account when assessing stability in the lab. Furthermore, the addition of drugs to NPs can alter the amount of surface charge on the NP, or disrupt the way that counter ions coordinate to the particle surface (termed the double layer). Kim et al. have demonstrated this by showing controllable aggregation with the addition of benzyl mercaptan to solutions of citrate coated Au NPs and have provided DLVO calculations by approximating ψ_0 as ζ to support their observations (Fig. 5) [33]. In addition, Ojea-Jimenez and Puentes have reported that cationic Au NPs are not electrostatically stable in the presence of a bridging anion (such as citrate) [34], and Segets et al. have analyzed the colloidal stability of negatively charged ZnO particles in different ionic strength solutions [35]. The loading of drugs via electrostatics may further decrease the effective charge of the NP which will diminish charge based stabilization.

2.2.3. Steric repulsion stabilization—Alternatively to charge based repulsion, the use of steric hindrance can effectively stabilize NP solutions in non-ideal conditions (i.e. high ionic strength). The organization of surfactants around the surface of the NP provides an effective “barrier” when inter-particle distances decrease, preventing aggregation [28]. The physical properties of these surfactants, however, will dictate how efficiently steric stabilization is utilized. Considerations of ligand flexibility (both in terms of stretching and compression), overall charge, and solubility are all key components to determining what will work in complex in vivo environments. Furthermore, the way that the ligand coating behaves is strongly related to the number of ligands per particle, termed the grafting density, and how much interaction there is between ligands. Generally, the more dense the grafting density the more extended the ligands are from the surface, forming the so called “brush configuration”. Kim and Matsen have provided theoretical assessments of the interaction between two polymer grafted particles, lending insight into how steric repulsion stabilizes NP suspensions [36]. Although thicker coatings (and thus increased inter particle distances) are desirable for stability, the saturation of the surface with ligands may present problems in how effective drug loading can be (i.e. less active sites and slower diffusion). In addition, triggers such as enzymes will also need to migrate through the ligand coating to cleave closely held drug bonds in covalent schemes, indicating that one must be careful to optimize the NP–drug conjugate both in terms of stability and final release.

In addition to the space available for binding on the surface of the NP, the actual surface curvature has recently highlighted as an important consideration in how polymer layers behave on NPs. Cederquist and Keating have noted that DNA orientation on NPs is highly dependent on the NP size [37], and Mei et al. found that the packing of PEG ligands on the surface of Au NPs was critical to the protection of the NP core from cyanide induced core decomposition [38]. Biver et al. utilized theory developed by Daoud and Cotton [40] to predict ligand thickness as a function of surface curvature on microparticles [39]. Recently, the application of this theory has been demonstrated for PEGylated Au NPs, with experimental results showing excellent agreement with calculations [20]. Fig. 6 shows the calculated polymer layer thickness for PEGylated Au NPs as a function of both particle size and ligand molecular weight with a constant grafting density (2.4 ligands/nm^2) [20]. Note that the insets depict a particle with the *same* hydrodynamic diameter but with different ligand thickness:particle core ratios. If one knows both (A) the relative attractive forces between two NPs (Eq. (1)) and (B) how steric repulsion behaves as a function of ligand corona properties [36], one can design non-aggregating particles which investigate the role of particle size in drug delivery. Understanding how soft coatings dictate biological interactions is an important direction for the future development of nanomedicine.

It is important to note that the nature of ligand structure of the surface of NPs can be dynamic. As an example, the use of thiol ligands on Au NPs is often thought to be quite stable, but Rotello and coworkers demonstrated that an exchange reaction involving glutathione could efficiently release a bound dye cargo [41]. Thus, while the NP carrier itself is often assumed to be static during transport, the issues accompanied with dynamic ligand–NP structure can play a major role in drug delivery.

2.2.4. Biological relevance of design considerations—Ultimately, a NP–drug conjugate must be stable in variable conditions within the body, where blood has a pH of 7.35–7.45 and an ionic strength of 150 mM. Moreover, a wide range of proteins can be found throughout the body, and these proteins have been shown to adsorb to NPs (Fig. 7) [42–44]. Based on these parameters, the use of electrostatic stabilization only is not sufficient for maintaining colloidal stability of NPs with high van der Waals attraction forces *in vivo*; ultimately these bare particles will eventually be coated with stabilizing ligands in the form of native biomolecules. In general, most NPs for *in vivo* applications are coated with a sterically shielding ligand layer which provides sufficient steric interactions to stabilize the conjugates. As seen from Eq. (1), the necessary properties will be dependent on both the material chosen and the size of the particle. Historically, the use of “stealth” ligands such as PEG have been important for reducing protein absorption and subsequent aggregation [45], but recently the use of shorter polyelectrolyte coatings has also been reported [14,46].

Following successful stabilization in blood environments, further consideration for the stability of NP–drug conjugates in harsher environments (such as cancer tissue with a lower pH [47]) should also be accounted for to limit unwanted cytotoxic effects. Pioneering work by Mattooussi et al. has explored the role of binding chemistry on the stability of PEGylated Au NPs [38], and their research has concluded that dithiol ligands provide better stability in harsh environments (high ionic strength and low pH) when compared to the monothiol analogs. Such work highlights the necessity for quantifying the physical properties of NPs and their potential effects on drug delivery. The future of nanotechnology will necessarily be the interface between the nanomaterial and surrounding media in biological, environmental, and technological applications.

3. Transport in biological environments

The practical use of nanometer size transport vectors can never be truly assessed using *in situ* experiments; it must always be tested in the increasingly complex environments of A) *in vitro* and B) *in vivo* test beds. The physical chemistry inherent to these new systems, however, makes quantitative understanding of how to rationally design NPs challenging. Here, we survey some of the relevant biophysical considerations for intravenous delivery of NP–drug conjugates, and review literature describing how the inherent physical properties of the NP might dictate efficacy. Moreover, we provide a critical review of how biomimetic hydrogel model systems can be used for a deeper understanding of NP behavior in complex media. Our discussion will focus on what physical properties of NPs determine efficacy, and examine how gel electrophoresis can aid in this endeavor [48].

3.1. Levels of complexity

The extrapolation from the highly controlled environment of a test tube to the intricacies of cellular interactions and transport of NPs *in vivo* is without question a daunting challenge. A survey of successful NP–drug delivery experiments over the last decade finds that they usually follow the same experimental trend: movement from the bench top to *in vitro* samples to model *in vivo* systems. The complications of living systems are both passive and active transport mechanisms, where the former relates to inherent physical properties

(diffusion, size, shape, etc.) and the later to specific biological processing of nanomaterials (i.e. macrophages, enzymatic destruction, etc.) [49]. Moreover, this simple categorization of processes is naïve, as both mechanisms are interconnected and dictate how the other operates. Here, we will limit our discussion to the physical chemistry at play in living systems, dividing the movement of NP-conjugates into three parts: intravenous transport, vasculature and tissue penetration, and cellular interactions. Through an exploration of recent literature, a broader understanding of the challenges facing the biological applications of NPs can be appreciated, and the importance of physical properties to effective drug delivery can be discerned.

3.1.1. Intravenous administration—The transition from in situ to in vivo for NP based drug delivery is usually characterized by injection of the NP solution either intravenously or directly to the area of interest [50]. The introduction of the conjugates to the blood stream is desirable for medical applications where the target is difficult to reach or has not yet been identified. Here, we consider some of the physical properties of NPs which dictate their transport through the vasculature system.

Immediately upon injection into the vasculature system, the NP–drug conjugate is immersed in a complex fluid composed of both high salt concentrations and numerous proteins which can adsorb onto the NP. These two situations can dramatically alter the stability of the NP as it circulates through the body; electrostatic repulsion is largely diminished due to high ionic strength, and the addition of proteins to the NP will alter both the hydrodynamic radius and particle charge [43]. From the standpoint of rational design, this is the reason why the NP must be sterically shielded from its biological environment by a soft coating [49]. Gref et al. has found that PEG coatings of >5 kDa molecular weight can effectively prevent significant protein adsorption [45]. Recently, Hamad et al. has found that the grafting density on the surface of the NP correlates with the degree of protein adsorption [51], which may provide additional degrees of freedom for designing viable NP–drug conjugates. Although this phenomenon is generally attributed to sterics, Moghimi and Szebeni caution that the concept of steric repulsion due to ligand coating alone may not suffice for the rational design of drug delivery NPs [52]. Rather one must take into account additional physical properties of the particle (including deformability, surface curvature, material, surface charge, and anchoring linkers) which also affect how the body responds to foreign particulates via an immune response known as complement activation [52,53].

The main mode of transport in the vascular system is bulk fluid movement (Fig. 8). Moghimi et al. provide an excellent introduction to this process in their review of drug delivery and the pharmacokinetics of nanometer sized delivery vectors [49]. The movement of fluid in the vascular system propels the NP–drug conjugate throughout the body, and the circulation of polymer coated Au NPs can be on the order of tens of hours [54]. This circulation is regulated by biological filters (including lymph nodes, kidneys, liver, etc.) which utilize both macrophages and size selective criteria for the filtering of foreign material [49,55]. Work by Bawendi, Fukumura and Jain has shown that particles with a hydrodynamic diameter (d_h) less than 5.5 nm can be cleared by the kidney [56], while others have demonstrated that larger particles undergo uptake by other filters [50]. A systematic study of NP properties and in vivo biodistribution was conducted by Choi et al. which showed that even slight changes in NP properties dramatically altered how it was processed in the body [57]. These critical observations provide an essential design criteria for NP based drug delivery: the hydrodynamic size and coating properties of a drug carrier often dictate its efficacy in vivo [46,58,59]. A small NP which was originally designed for renal clearance may accumulate a variety of proteins and thus may no longer be viable for the renal pathway; conversely a particle which has been designed to limit protein adsorption but has large hydrodynamic radius may be too large for effective clearance [46].

3.1.2. Tissue and vasculature penetration—The movement of the NP–drug conjugate into the tissue has been studied in detail, especially for diseases which involve large inflammatory responses such as cancer and solid tumors [49]. In these conditions, the epithelial cells which line the nearby vasculature are less ordered than healthy tissue, and allow for the trapping of NPs by the local matrix (Fig. 8). In addition, ineffective fluid removal from solid tumors results in accumulation of NP drug carriers, and the process has been accordingly labeled the enhanced permeability and retention effect (EPR) [9]. Maeda notes that this process is “the gold standard” for solid tumor therapy and notes that below a finite molecular weight (approximately 80 kDa) no favorable partitioning in tumor tissue is observed compared to healthy tissue [60]. This process has been utilized by many groups for NP mediated therapy where the NP itself can be the active participant, or a molecular therapeutic is released in the tumor itself [5,9,57].

The transition from a relatively hindrance free environment to that of an anisotropic and dense matrix presents a further challenge to NP mediated drug delivery. Seminal work by Bawendi, Fukumura, and Jain has detailed this process by imaging the movement of quantum dots from the vascular system to tumors, including diffusion through the extracellular matrix [61]. Recently, a novel experiment by the same authors detailed the accumulation of large NP-gelatin hybrids (≈ 100 nm d_h) which could be subsequently degraded via excess matrix metalloproteinases (MMPs) to release the encapsulated QDs (≈ 10 nm d_h), highlighting that EPR designed particles have hindered transport through the tumor in comparison to smaller particles (Fig. 9) [62]. Interestingly, Perrault et al. have studied particles with intermediate hydrodynamic diameters (20–80 nm) and have observed that although the diffusional process is severely hindered, particles with $d_h < 40$ nm can translate from the vasculature into tumor tissue (Fig. 10) [54]. Similar phenomena are observed for biofilms [63], movement from airspaces to lymph nodes [64], and to some degree in diseases resulting in excess mucus accumulation (such as cystic fibrosis) [65,66]. The addition of steric complications (which is negligible for small molecules) can hinder the movement of NPs into deep tissues and thus only allows for local drug release.

3.1.3. Penetrating cell membranes—Once the NP has translated the dense matrix which surrounds cells, the drug vector must successfully interact with the cellular target to initiate drug uptake. The association of NPs with cells has been studied extensively [67,42,68–73]. In general, negatively charged or neutral particles tend to be repelled by the cellular membrane, and undergo uptake via facilitated transport [69]. Positively charged particles, on the other hand, can interact with the outer cell membrane and can permeate into the cell [74]. These simple observations are complicated by the existence of other exterior cell components (macrophages, extracellular proteins, enzymes, etc.) which are used to identify and regulate foreign material as noted by Nel et al. [75]. Chan and co-workers have recently provided an extensive discussion of how NPs interact with cells, noting that targeting with cellular receptors can further enhance association [74].

Whether the uptake of the NPs is desirable from a medical point of view depends on the methodology used for drug delivery and the targeted therapy mechanism. Certain drugs become only effective once incorporated into the cell and in select organelles [76,77]. The direct incorporation of NPs into the cells, however, may provoke unwanted side effects including hindered clearance and adverse cell behavior [75]. Conversely, the design of a NP to interact with a cell but not penetrate it may also hinder clearance from the body if this is achieved via steric interactions [46]. In light of this, it is important to identify the final desired outcome at the cellular level, and to determine how this outcome affects the eventual clearance from the body. Furthermore, one needs to consider what drug delivery mechanism should be employed to maximize efficacy and minimize unwanted cytotoxicity.

3.2. Quantitative physical considerations and non-covalent drug delivery

The challenge for NP mediated drug delivery, then, is to provide an optimized system which takes advantage of each stage of delivery in vivo. This optimization can only occur with an understanding of what physical properties of the NP dictate its biological transport. Moreover, what happens to the drug during transport has also been absent in the discussion thus far. Here, we quickly review the major contributing physical parameters of bulk flow transport, steric effects, and electrostatics which dictate efficacy at each individual stage, and discuss the relevant effect on non-covalent drug delivery.

3.2.1. Bulk flow transport—The main physical transport mechanism in the body is the use of bulk flow transport through the mechanical pumping associated with circulatory system. The movement of blood throughout the body results in a systematic current which rapidly sweeps the NP–drug conjugates throughout the body. The hydrodynamic diameter and relative size of the particle will dictate how efficiently this process occurs. Jain and Stylianopoulos provide equations describing the movement of nanoparticles both A) in the blood vessels and B) into the nearby tissue, concluding that both the physical properties of the particle and the media dictate NP transport [78]. The systematic evaluation of NP movement through the vascular system with diffusion out of the vasculature has been provided by Gentile et al. [79]. The interaction with other blood components (proteins, enzymes, cell membranes, endothelial walls) provides a further complication to the movement of NPs. In addition, Moghimi et al. state that the phenomenon of drug pooling also occurs in the vasculature [49], and this is expected to influence NP mediated drug delivery as well. Although both the EPR effect and the desired stealth character can be obtained with large polymer coatings, Jain and Stylianopoulos are quick to note that smaller particles will have better diffusion and convection in tumor tissue [78]. This leads to the design of a NP–drug conjugate which is either A) the local minimum of size which optimizes the EPR effect and diffusion/convection [54,75], or B) utilizes a two stage approach in delivery (as described by Bawendi, Jain, and Fukumura) [62].

How does bulk transport affect the non-covalently loaded drug payload? If the remaining particles contain the drug inside (as in liposomes, micelles, or mesoporous silica NPs), it is expected that, as with covalently bound drugs, little change in drug characteristics is observable (though how efficient drug delivery is upon reaching tumor cells is still problematic) [59]. With the surface mediated non-covalently bound drug, however, contact with other serum constituents, deformations during vascular escape, and the change in ligand coating associated with a two-stage NP delivery system all might play a role in how well NP–drug conjugates are delivered.

3.2.2. Steric effects—The steric effects of NP–drug conjugates are a critical consideration with designing better drug delivery vectors. In terms of stabilization, the use of steric repulsion is the preferential way for maintaining colloidal properties. However, these same steric forces will also determine how well the NP migrates through tissue, approaches cell membranes, and whether the particle can be cleared from the body [46,56]. The two primary sources of steric forces present in any NP system include A) the NP core and B) the polymer coating; the relative contribution of each to the overall interaction between the NP and the local matrix will necessarily be dependent on the ratio of ligand thickness to core radius and the local environment. Moreover, the ligand corona flexibility provides a further parameter which determines whether the particle shape can be deformed as it moves through tissue. Recently, Merkel et al. found that red blood cell mimics (RBCMs) which were highly deformable had much better circulation time and more favorable bio-distribution in in vivo studies [80]. While the RBCMs were microparticles, the same considerations also apply to NPs as demonstrated by Stylianopoulos et al. for the

diffusion through tissue [61]. Recently, Cheng et al. found that a PDT drug could be delivered deep into the tumor of mice as monitored by fluorescence imaging, and found that after 4 h a significant amount of Au NPs could be found in tumor tissue [6]. Although the microscopic location of the Au within the tumor tissue was not reported, their previous release mechanism of either membrane permeation by the drug (99%) or endocytosis of the Au NP–drug carrier (1%) seems to imply that the particle was able to penetrate through the tissue [25]. Given that the data was collected 4 h after injection and the d_h is roughly 40 nm (which were shown to diffuse into tumor tissue by Perrault et al. [54]) this seems plausible, even though data collected by Ramanujan et al. indicate that the process is severely hindered [81]. Future work for NP-mediated drug delivery must establish whether these steric hindrances are significant within a given therapeutic time window.

For covalent NP–drug conjugates, it may be expected that the bio-distribution of both the NP and the drug are relatively the same, but for non-covalent systems this may not be the case. Cheng et al. found that the fluorescence from the non-covalently loaded drug payload did not have a direct correspondence to where the Au NPs were found after graphite furnace atomic absorption spectroscopy (GFAAS) was conducted for each critical organ (Fig. 11). Although they found that the uptake and clearance profile for the tumor of both the drug and Au NPs were similar, the drug fluorescence was heavily located in the tumor, stomach and large intestine (Fig. 11A) while the Au NPs were found mostly in the spleen, kidneys, lungs, and urinary tract (Fig. 11B). This work highlights an important aspect of non-covalent drug delivery: even with successful loading and targeting of the NPs, subsequent uptake of the drug at one specific location is not necessarily ensured. For drugs with documented side effects (such as doxorubicin), careful consideration for the chance of unwanted side effects should be given. As the drug in Fig. 11 is only active under exposure to high fluence 670 nm light, [6] no cytotoxic effects were observed during this study, but the data serves as a reminder that caution is needed when designing a NP–drug therapeutic.

3.2.3. Electrostatics—The final consideration for optimizing drug delivery using NPs is the relative charge of the NP itself. In addition to cellular uptake [74], the incorporation of electrostatic charges for the modification of particle–matrix interactions has also been shown to be important for the movement of particles [61,78]. When incorporating a drug payload onto a charged NP, it can be a challenge to discern how perturbations to the electrostatics will affect the overall transport properties. Moreover, it has been proposed that the generation of local electrical fields by cells may also affect charged molecular transport [82]. Robinson and Messerli have stated that slight changes in nearby cell membrane potentials on the order of a few mV can generate electric fields on the order of 10^5 mV/mm⁻¹ [82]. Moreover, Glaser notes that the proliferation rates of cells are correlated to their overall membrane potential, with proliferating cells having lower potentials (–10 to –30 mV) and stationary cells having larger potentials (–70 to –90 mV) [83]. These electrostatic considerations could lead to a better understanding of how NP transport in extracellular fluid can be further controlled.

These same considerations which we have applied to the NP must also be applied to the drug payload. It has recently been demonstrated that a cationic and hydrophobic drug readily undergoes uptake through negative cell membranes and thus can be delivered without direct cell uptake of the NP [25]. A critical question in this process, however, is at what distance the potential of the cell may influence a stabilized drug, and how the surface potential of a NP compares with the surface potential of a nearby cell membrane. Moreover, do charges within the protective ligand layer around the NP (known as polyelectrolyte coatings) effect the way in which charged drugs migrate to cells? In looking at experiments conducted by Kim et al., the use of zwitterionic charges seems to have little effect on drug loading and

release [14]. However, when the electrostatics are used for drug incorporation and stabilization, these additional charges may hinder the overall loading efficiency.

3.3. Agarose gel electrophoresis as a model system

The assessment of the physical properties of NP often must take a modular approach, as it can be difficult to separate the physical and active biological mechanisms inherent to both in vitro and in vivo experiments. One methodology which has received extensive treatment in the literature is the use of biomimetic model systems. These have mainly focused on polysaccharides such as collagen (negatively charged) and hyaluronic acid (positively charged), which are main constituents of biologically relevant matrices [78]. Interestingly, the use of polysaccharide hydrogels has a long history in biology through the development of agarose based gel electrophoresis and subsequent quantification of gel parameters [84–87]. In the field of nanotechnology, this technique has been utilized to quantify DNA binding [88], different sizes and shapes [89], and grafting density [90]. Recently, the use of agarose gel electrophoresis for the quantification of the physical properties of NPs has been reported [48]. Here, we critically evaluate how the physical properties examined in agarose gel electrophoresis can be utilized for rational design in biomedical applications, and how future modifications to the technique might provide a modular approach to biomimetic models.

3.3.1. Probing electrostatics—In the experiment reported by Doane et al., Au NPs with an average core diameter of 6 nm were coated with HS-mPEG of varying molecular weight (1 kDa, 2 kDa, 5 kDa, and 10 kDa) and these particles were subsequently analyzed via agarose gel electrophoresis [48]. As was previously reported, the mobilities of the Au NPs varied as a function of PEG molecular weight (Fig. 12), with the 1 kDa capped particles migrating towards the positive electrode, the 2 kDa effectively not moving, and both the 5 and 10 kDa particles migrating towards the negative electrode. Previously this had been thought to indicate a change in particle charge due to increasing incorporation of PEG [90]; however, work by Perrault and Chan showed that these particles had consistently negative ζ -potentials [91]. Through numerous variations to the experimental procedure, Doane et al. were able to show that two distinct forces were at play during electrophoresis, namely the electromigration of the charged particles and the counterforce of electroosmosis. Due to the migration of the neutral vitamin B12 marker [89], it was determined that the negative charge on the matrix played a critical role in developing a bulk movement of fluid which effectively dragged the particles (which still were observed to have a very small negative ζ -potential) towards the negative electrode.

Interestingly, follow up work indicated that with proper assessment of both Au NP and PEG ligand corona properties, a net negative charge could be deduced from ζ -potential measurements on the order of -50 to -60 mV [20]. Thus not only does the particle migrate in an electric field, it may also be important for the stabilization of protonated drugs. Several authors have reported that attaching different functionalities to the ends of PEG ligands ($-\text{COOH}$, $-\text{NH}_2$, etc.) can effectively control the particle migration in an electric field [89], indicating that the incorporation of charged ligands will have an important effect on how particles migrate in charged matrices as shown by Stylianopoulos et al. [61].

3.3.2. Understanding steric frictional forces—The electrostatics for mPEG-coated Au NPs, however, is decidedly less important for heavier PEG molecular weights (and thus thicker polymer coatings), as indicated by the reversed mobility of the 5 kDa and 10 kDa species (Fig. 12). Theoretical calculations by coauthor Hill established previously unknown frictional coupling coefficients describing the interaction between the PEG chains and the local agarose fibers [48]. Building off of previous theoretical calculations for soft particles

(core particles with soft polymer shells) [92–94], the relative role of the polymer coating in both the susceptibility to electroosmosis and the frictional drag due to interactions with local fibers could be discerned. Such behavior was supported by observing the mobility of the Au NPs with varying agarose concentrations [48].

Interestingly, when a similar experiment was conducted with epidermal growth factor (EGF) targeted Au NPs both with and without a drug payload [95], significant differences were seen between the loaded and unloaded Au NPs (Fig. 13). Although the precursor particles included bifunctional PEG with negatively charged carboxyl groups, their effect on the overall mobility of the as-synthesized NPs was limited due to both their short chain length (3 kDa) and relatively low abundance (10%). The conjugation of the EGF peptide resulted in farther migration to the negative electrode, which may be expected both to decreased negative charge and an overall larger coating. However, although it may be expected that the addition of a protonated drug would cause the particle to move *farther* to the negative electrode, the observed result was actually a *decreased* mobility. Preliminary evidence would suggest that the addition of a hydrophobic drug to the PEG corona modifies how it behaves in the complex agarose matrix. Further experiments are necessary to assess how drug payloads modify particle properties and dictate their transport through dense matrices such as tissue.

3.3.3. Development of rational design—The properties discerned via gel electrophoresis are important considerations for how NPs move in living systems. While it should be clear that the term “biomimetic” in this instance does not mean a direct correlation to a living system, the inherent physics of particle–ligand behavior and particle–matrix interactions can be explored apart from active transport mechanisms. Based on the discussion of agarose gel electrophoresis, it cannot only be concluded that 1) bulk fluid movement can dramatically alter NP mobility, 2) steric effects can alter NP transport, and 3) NP charge can play a role in particle migration, but more importantly that these properties can be quantified. The use of a modular approach also allows for the possibility for different experimental design (forced volume vs. electric fields, different hydrogel type, different ionic strengths, buffer constituents, addition of common extracellular proteins, etc.) which can further establish how NPs passively interact with biological matrices.

Using such models and rigorous control of the experimental parameters, the screening of potential NP candidates based on fundamental physical properties can help to establish design criteria for better NP-based therapeutics. While a complete understanding of the surrounding environment may be unobtainable given the diversity and complexity of biological systems, understanding how the NP behaves in varying systems can lead to smarter and more efficient NP design. Recently, Fourches et al. have presented a quantitative nanostructure activity relationship (QNAR) concept which aims to effectively screen NPs based both on physical and chemical properties and in vitro data assessment [96]. In light of the pioneering work for vasculature transport, tissue diffusion, and cell interactions for in vivo applications, similar criteria will continue to be developed for NP based drug delivery based on inherent physical properties [58,59,97,98]. Understanding which fundamental physical forces are dominant in NP transport are vital for realizing the potential of nanomedicine, and improving already commercialized drug delivery techniques (i.e. liposomes, micelles, etc.) Although the last decade has brought significant progress in both the design of biologically designed NPs and the understanding of intricate diseases, it is clear that the additional identification of physiological considerations will greatly enhance future therapeutic initiatives.

4. Non-covalent drug release and cellular uptake

After careful loading and successful transport through the body, the next step for NP-mediated drug delivery is the delivery of the therapeutic payload. Efficient release of the drug from the NP–drug conjugate to the targeted cell results from either A) chemical reactions or B) changes in the thermodynamic stability of the associated drug. While covalently bound drugs require some sort of bond cleavage (i.e. phototriggered release, thermal bond breakage, bond reduction, ligand exchange etc.), non-covalently bound drugs on the surface of NPs require a change in local physical forces. Hydrophobic drugs, for example, are released due to the change in local hydrophobicity, inducing drug diffusion from the NP to the local biological membranes. Thus while non-covalently delivered drugs are much simpler to release, they also have the potential for unwanted non-specific uptake. In contrast, many covalently bound systems have low non-specific release but require an external factor (i.e. high concentrations of enzymes, light, pH, thermal energy, etc.) to release the payload. The continuing challenge is to develop NP-conjugates which are stable during transport but deliver their payload efficiently upon arriving at the target.

Subsequent release of the drug requires efficient uptake by the target cells, and these mechanisms have been explored in detail [99,100]. In general, however, the NP should be as close to the cell as possible to limit diffusion away from the target, uptake by non-targeted cells, and to prevent possible drug aggregation. The properties of the NP, then, must allow for localization in or near the cell, and for kinetically favorable diffusion of the drug to the target. In general, NP based drug delivery vectors target the inside of the cell [41,101,102], or closely associate with outer cell membranes [25,14]. In either case, effective delivery must thermodynamically favor drug release versus stabilization of the conjugate and kinetically must be fast enough upon reaching the target.

Several examples of improved therapy due to NP transport are reported in the literature. Ferris et al. have shown that the use of mesoporous silica nanoparticles (MSNs) for the delivery of hydrophobic drugs dramatically improves cell killing in cancer cell lines [102]. Fig. 14 details how camptothecin loaded MSNs (Fig. 14A shows the general schematic) effectively double the therapeutic effect compared to the free drug. Moreover, when the authors utilized a targeting peptide, the efficacy was increased nearly 3 times (Fig. 14B) [102]. Kester et al. found that using calcium phosphate NPs (CPNPs) could greatly increase the amount of the hydrophobic drug ceramide in melanoma cells when compared to the drug by itself in dimethylsulfoxide (DMSO) [17]. The encapsulated drug is sterically trapped within the particle core at physiological pH (7.4), and is subsequently dissolved in more acidic media (pH<6.5). Moreover, the authors utilized amines to yield positively charged particles ($\zeta=+30$ mV) for interaction with cell membranes and subsequent internalization. With surface mediated non-covalent drug delivery methodology, Cheng et al. found that the uptake of a PDT drug in vivo dramatically increased when the drug was conjugated to PEGylated particles [5], and when a targeting ligand was attached, could even deliver the drug across the blood–brain-barrier [95]. Recently, Kim et al. have shown that the incorporation of several hydrophobic molecules on zwitterionic Au NPs led to efficient drug release in human breast cancer cells [14].

These examples illustrate why nanomaterials have received so much attention for drug delivery: more drug uptake per dose and the potential for higher specificity in targeting select diseases. Recently, Ferris et al. have noted that 40% of all new drugs emerging from the pharmaceutical pipeline are hydrophobic, [102] which will require an effective transporter in order to maximize efficacy. Despite the complexities of stability and in vivo transport, the prospect of using highly controlled NPs continues to drive the field of noncovalent drug delivery forward.

5. Summary

During the last decade, nanomedicine has become a promising approach for the selective and efficient treatment of human diseases. Without a proper understanding of the physical chemistry involved in loading, transporting, and delivering drugs with NP vectors, however, development of successful therapies will be significantly slowed. In this review we have discussed several of the primary forces involved at each stage of NP–drug delivery development, highlighting the significant progress which has been achieved. Future work within the field will continue to determine which NP physical properties are critical for rationally designed drug delivery, subsequently improving design criteria and enabling NPs to reach their considerable potential. At this point in time, it is apparent that both covalent and non-covalent drug deliveries are being developed as independent techniques. The utilization of one over the other may be preferable depending on the disease type and tissue target of the vector. However, the fundamental physical properties of the particles will continue to influence efficacy, and must be better understood to move the field of nanomedicine forward.

References

1. Burda C, Chen X, Narayanan R, El-Sayed MA. Chemistry and properties of nanocrystals of different shapes. *Chem Rev.* 2005; 105:1025–1102. [PubMed: 15826010]
2. Boal AK, Rotello VM. Fabrication and self-optimization of multivalent receptors on nanoparticle scaffolds. *J Am Chem Soc.* 2000; 122:734–735.
3. Murray CB, Norris DJ, Bawendi MG. Synthesis and characterization of nearly monodisperse Cde (e = sulfur, selenium, tellurium) semiconductor nanocrystallites. *J Am Chem Soc.* 1993; 115:8706–8715.
4. Kim BYS, Rutka JT, Chan WCW. Nanomedicine. *N Engl J Med.* 2010; 363:2434–2443. [PubMed: 21158659]
5. Cheng Y, Samia AC, Meyers JD, Panagopoulos I, Fei B, Burda C. Highly efficient drug delivery with gold nanoparticle vectors for in vivo photodynamic therapy of cancer. *J Am Chem Soc.* 2008; 130:10643–10647. [PubMed: 18642918]
6. Cheng Y, Meyers JD, Broome AM, Kenney ME, Basilion JP, Burda C. Deep penetration of a PDT drug into tumors by noncovalent drug–gold nanoparticle conjugates. *J Am Chem Soc.* 2011; 133:2583–2591. [PubMed: 21294543]
7. Karshikoff, A. *Non-covalent Interactions in Proteins.* Imperial College Press; London: 2006.
8. Lavik E, von Recum H. The role of nanomaterials in translational medicine. *ACS Nano.* 2011; 5:3419–3424. [PubMed: 21604811]
9. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics. A review. *J Control Release.* 2000; 65:271–284. [PubMed: 10699287]
10. Duncan B, Kim C, Rotello VM. Gold nanoparticle platforms as drug and biomacromolecule delivery systems. *J Control Release.* 2010; 148:122–127. [PubMed: 20547192]
11. Kelkar SS, Reineke TM. Theranostics: combining imaging and therapy. *Bioconjug Chem.* 2011; 22:1879–1903. [PubMed: 21830812]
12. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotechnol.* 2007; 2:751–760. [PubMed: 18654426]
13. Lu J, Liong M, Zink JJ, Tammanoi F. Mesoporous silica nanoparticles as a delivery system for hydrophobic anticancer drugs. *Small.* 2007; 3:1341–1346. [PubMed: 17566138]
14. Kim CK, Ghosh P, Pagliuca C, Zhu ZJ, Menichetti S, Rotello VM. Entrapment of hydrophobic drugs in nanoparticle monolayers with efficient release into cancer cells. *J Am Chem Soc.* 2009; 131:1360–1361. [PubMed: 19133720]
15. Manju S, Sreenivasan K. Enhanced drug loading on magnetic nanoparticles by layer-by-layer assembly using drug conjugates: blood compatibility evaluation and targeted drug delivery in cancer cells. *Langmuir.* 2011; 27:14489–14496. [PubMed: 21988497]

16. Kim BS, Park SW, Hammond PT. Hydrogen-bonding layer-by-layer-assembled biodegradable polymeric micelles as drug delivery vehicles from surfaces. *ACS Nano*. 2008; 2:386–392. [PubMed: 19206641]
17. Kester M, Heakal Y, Fox T, Sharma A, Robertson GP, Morgan TT, Altinoglu EI, Tabakovic A, Parette MR, Rouse S, Ruiz-Velasco V, Adair JH. Calcium phosphate nanocomposite particles for in vitro imaging and encapsulated chemotherapeutic drug delivery to cancer cells. *Nano Lett*. 2008; 8:4116–4121. [PubMed: 19367878]
18. Morgan TT, Muddana HS, Altinoglu EI, Rouse SM, Tabakovic A, Tabouillot T, Russin TJ, Shanmugavelandy SS, Butler PJ, Eklund PC, Yun JK, Kester M, Adair JH. Encapsulation of organic molecules in calcium phosphate nanocomposite particles for intracellular imaging and drug delivery. *Nano Lett*. 2008; 8:4108–4115. [PubMed: 19367837]
19. Rosenholm JM, Peuhu E, Eriksson JE, Sahlgren C, Linden M. Targeted intracellular delivery of hydrophobic agents using mesoporous hybrid silica nanoparticles as carrier systems. *Nano Lett*. 2009; 9:3308–3311. [PubMed: 19736973]
20. Doane TL, Chuang CH, Hill RJ, Burda C. Nanoparticle ζ -potentials. *Acc Chem Res*. 2012; 45:317–326. [PubMed: 22074988]
21. Lucarini M, Franchi P, Pedulli GF, Pengo P, Scrimin P, Pasquato L. EPR study of dialkyl nitroxides as probes to investigate the exchange of solutes between the ligand shell of monolayers of protected gold nanoparticles and aqueous solutions. *J Am Chem Soc*. 2004; 126:9326–9329. [PubMed: 15281823]
22. Lucarini M, Franchi P, Pedulli GF, Gentilini C, Polizzi S, Pengo P, Scrimin P, Pasquato L. Effect of core size on the partition of organic solutes in the monolayer of water-soluble nanoparticles: an ESR investigation. *J Am Chem Soc*. 2005; 127:16384–16385. [PubMed: 16305211]
23. Andersson J, Rosenholm J, Areva S, Linden M. Influences of material characteristics on ibuprofen drug loading and release profiles from ordered micro- and mesoporous silica matrices. *Chem Mater*. 2004; 16:4160–4167.
24. Lee CH, Lo LW, Mou CY, Yang CS. Synthesis and characterization of positive-charge functionalized mesoporous silica nanoparticles for oral drug delivery of an anti-inflammatory drug. *Adv Funct Mater*. 2008; 18:3283–3292.
25. Cheng Y, Samia AC, Li J, Kenney ME, Resnick A, Burda C. Delivery and efficacy of a cancer drug as a function of the bond to the gold nanoparticle surface. *Lang-muir*. 2010; 26:2248–2255.
26. Lee BS, Park K, Park S, Kim GC, Kim HJ, Lee S, Kil H, Oh SJ, Chi D, Kim K, Choi K, Kwon IC, Kim SY. Tumor targeting efficiency of bare nanoparticles does not mean the efficacy of loaded anticancer drugs: Importance of radionuclide imaging for optimization of highly selective tumor targeting polymeric nanoparticles with or without drug. *J Control Release*. 2010; 147:253–260. [PubMed: 20624433]
27. Cho EC, Zhang Q, Xia Y. The effect of sedimentation and diffusion on cellular uptake of gold nanoparticles. *Nat Nanotechnol*. 2011; 6:385–391. [PubMed: 21516092]
28. Hunter, RJ. *Foundations of Colloid Science*. Vol. 1. Oxford University Press; Oxford: 1987.
29. Cosgrove, T. *Colloid Science: Principles, Methods and Applications*. 2. Wiley; New York: 2010.
30. Russel, WB.; Saville, DA.; Schowalter, WR. *Colloidal Dispersions*. Cambridge University Press; New York: 1989.
31. Bergstrom L. Hamaker constants of inorganic materials. *Adv Colloid Interface Sci*. 1997; 70:125–169.
32. Demirci S, Enustun BV, Turkevich J. Stability of colloidal gold and determination of the Hamaker constant. *J Phys Chem*. 1978; 82:2710–2711.
33. Kim T, Lee K, Gong MS, Joo SW. Control of gold nanoparticle aggregates by manipulation of interparticle interaction. *Langmuir*. 2005; 21:9524–9528. [PubMed: 16207031]
34. Ojea-Jimenez I, Puentes V. Instability of cationic gold nanoparticle bioconjugates: the role of citrate ions. *J Am Chem Soc*. 2009; 131:13320–13327. [PubMed: 19711893]
35. Segets D, Marczak R, Schafer S, Paula C, Gnichwitz JF, Hirsch A, Peukert W. Experimental and theoretical studies of the colloidal stability of nanoparticles—a general interpretation based on stability maps. *ACS Nano*. 2011; 5:4658–4669. [PubMed: 21545143]

36. Kim JU, Matsen MW. Interaction between polymer-grafted particles. *Macromolecules*. 2008; 41:4435–4443.
37. Cederquist KB, Keating CD. Curvature effects in DNA: Au nanoparticle conjugates. *ACS Nano*. 2009; 3:256–260. [PubMed: 19236058]
38. Mei BC, Oh E, Susumu K, Farrell D, Mountziaris TJ, Mattoussi H. Effects of ligand coordination number and surface curvature on the stability of gold nanoparticles in aqueous solutions. *Langmuir*. 2009; 25:10604–10611. [PubMed: 19588955]
39. Biver C, Hariharan R, Mays J, Russel WB. Neutral and charged polymer brushes: a model unifying curvature effects from micelles to flat surfaces. *Macromolecules*. 1997; 30:1787–1792.
40. Daoud M, Cotton JP. Star shaped polymers: a model for the conformation and its concentration dependence. *J Phys*. 1982; 43:531–538. (France).
41. Hong R, Han G, Fernandez JM, Kim BJ, Forbes NS, Rotello VM. Glutathione-mediated delivery and release using monolayer protected nanoparticle carriers. *J Am Chem Soc*. 2006; 128:1078–1079. [PubMed: 16433515]
42. Chithrani BD, Ghazani AA, Chan WCW. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Lett*. 2006; 6:662–668. [PubMed: 16608261]
43. Dobrovolskaia MA, Patri AK, Zheng J, Clogston JD, Ayub N, Aggarwal P, Neun BW, Hall JB, McNeil SE. Interaction of colloidal gold nanoparticles with human blood: effects on particle size and analysis of plasma protein binding profiles. *Nanomedicine*. 2009; 5:106–117. [PubMed: 19071065]
44. Albanese A, Chan WCW. Effect of gold nanoparticle aggregation on cell uptake and toxicity. *ACS Nano*. 2011; 5:5478–5489. [PubMed: 21692495]
45. Gref R, Luck M, Quellec P, Marchand M, Dellacherie E, Harnisch S, Blunk T, Muller RH. ‘Stealth’ corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. *Colloids Surf, B*. 2000; 18:301–313.
46. Choi HS, Liu W, Liu F, Nasr K, Misra P, Bawendi MG, Frangioni JV. Design considerations for tumor-targeted nanoparticles. *Nat Nanotechnol*. 2010; 5:42–47. [PubMed: 19893516]
47. Helmlinger G, Yuan F, Dellian M, Jain RK. Interstitial pH and pO₂ gradients in solid tumors in vivo: high-resolution measurements reveal a lack of correlation. *Nat Med*. 1997; 3:177–182. [PubMed: 9018236]
48. Doane T, Babar A, Cheng Y, Hill RJ, Burda C. Electrophoretic mobilities of PEGylated gold NPs. *J Am Chem Soc*. 2010; 132:15624–15631. [PubMed: 20958038]
49. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. *Pharmacol Rev*. 2001; 53:283–318. [PubMed: 11356986]
50. Lipka J, Semmler-Behnke M, Sperling RA, Wenk A, Takenaka S, Schleh C, Kissel T, Parak WJ, Kreyling WG. Biodistribution of PEG-modified gold nanoparticles following intratracheal instillation and intravenous injection. *Biomaterials*. 2010; 31:6574–6581. [PubMed: 20542560]
51. Hamad I, Al-Hanbali O, Hunter AC, Rutt KJ, Andresen TL, Moghimi SM. Distinct polymer architecture mediates switching of complement activation pathways at the nanosphere–serum interface: implications for stealth nanoparticle engineering. *ACS Nano*. 2010; 4:6629–6638. [PubMed: 21028845]
52. Moghimi SM, Szebeni J. Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties. *Prog Lipid Res*. 2003; 42:463–478. [PubMed: 14559067]
53. Moghimi SM, Andersen AJ, Ahmadvand D, Wibroe PP, Andresen TL, Hunter AC. Material properties in complement activation. *Adv Drug Deliv Rev*. 2011; 63:1000–1007. [PubMed: 21689701]
54. Perrault SD, Walkey C, Jennings T, Fischer HC, Chan WCW. Mediating tumor targeting efficiency of nanoparticles through design. *Nano Lett*. 2009; 9:1909–1915. [PubMed: 19344179]
55. Kim S, Lim YT, Soltesz EG, De Grand AM, Lee J, Nakayama A, Parker JA, Mihaljevic T, Laurence RG, Dor DM, Cohn LH, Bawendi MG, Frangioni JV. Near-infrared fluorescent type ii quantum dots for sentinel lymph node mapping. *Nat Biotechnol*. 2004; 22:93–97. [PubMed: 14661026]

56. Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Ipe BI, Bawendi MG, Frangioni JV. Renal clearance of quantum dots. *Nat Biotechnol.* 2007; 25:1165–1170. [PubMed: 17891134]
57. Choi HS, Ipe BI, Misra P, Lee JH, Bawendi MG, Frangioni JV. Tissue- and organ-selective biodistribution of NIR fluorescent quantum dots. *Nano Lett.* 2009; 9:2354–2359. [PubMed: 19422261]
58. Alexis F, Pridgen E, Molnar LK, Farokhzad OC. Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Mol Pharm.* 2008; 5:505–515. [PubMed: 18672949]
59. Li SD, Huang L. Pharmacokinetics and biodistribution of nanoparticles. *Mol Pharm.* 2008; 5:496–504. [PubMed: 18611037]
60. Maeda H. The enhanced permeability and retention (epr) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. *Adv Enzyme Regul.* 2001; 41:189–207. [PubMed: 11384745]
61. Stylianopoulos T, Poh MZ, Insin N, Bawendi MG, Fukumura D, Munn LL, Jain RK. Diffusion of particles in the extracellular matrix: the effect of repulsive electrostatic interactions. *Biophys J.* 2010; 99:1342–1349. [PubMed: 20816045]
62. Wong C, Stylianopoulos T, Cui J, Martin J, Chauhan VP, Jiang W, Popovic Z, Jain RK, Bawendi MG, Fukumura D. Multistage nanoparticle delivery system for deep penetration into tumor tissue. *Proc Natl Acad Sci U S A.* 2011:1–6.
63. Peulen TO, Wilkinson KJ. Diffusion of nanoparticles in a biofilm. *Environ Sci Technol.* 2011; 45:3367–3373. [PubMed: 21434601]
64. Choi HS, Ashitate Y, Lee JH, Kim SH, Matsui A, Insin N, Bawendi MG, Semmler-Behnke M, Frangioni JV, Tsuda A. Rapid translocation of nanoparticles from the lung airspaces to the body. *Nat Biotechnol.* 2010; 28:1300–1303. [PubMed: 21057497]
65. Lai SK, Wang YY, Hanes J. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. *Adv Drug Deliv Rev.* 2009; 61:158–171. [PubMed: 19133304]
66. Cone RA. Barrier properties of mucus. *Adv Drug Deliv Rev.* 2009; 61:75–85. [PubMed: 19135107]
67. Connor EE, Mwamuka J, Gole A, Murphy CJ, Wyatt MD. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small.* 2005; 1:325–327. [PubMed: 17193451]
68. Chithrani BD, Chan WCW. Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. *Nano Lett.* 2007; 7:1542–1550. [PubMed: 17465586]
69. Jiang W, Kim BYS, Rutka JT, Chan WCW. Nanoparticle-mediated cellular response is size-dependent. *Nat Nanotechnol.* 2008; 3:145–150. [PubMed: 18654486]
70. Verma A, Uzun O, Hu Y, Hu Y, Han HS, Watson N, Chen S, Irvine DJ, Stellacci F. Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles. *Nat Mater.* 2008; 7:588–595. [PubMed: 18500347]
71. Cho EC, Xie J, Wurm PA, Xia Y. Understanding the role of surface charges in cellular adsorption versus internalization by selectively removing gold nanoparticles on the cell surface with a I₂/KI etchant. *Nano Lett.* 2009; 9:1080–1084. [PubMed: 19199477]
72. Albanese A, Sykes EA, Chan WCW. Rough around the edges: the inflammatory response of microglial cells to spiky nanoparticles. *ACS Nano.* 2010; 4:2490–2493. [PubMed: 20496953]
73. Moyano DF, Rotello VM. Nano meets biology: structure and function at the nanoparticle interface. *Langmuir.* 2011; 27:10376–10385. [PubMed: 21476507]
74. Chou LYT, Ming K, Chan WCW. Strategies for the intracellular delivery of nanoparticles. *Chem Soc Rev.* 2011; 40:233–245. [PubMed: 20886124]
75. Nel AE, Madler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, Klaessig F, Castranova V, Thompson M. Understanding biophysicochemical interactions at the nano-bio interface. *Nat Mater.* 2009; 8:543–557. [PubMed: 19525947]
76. Nori A, Kopecek J. Intracellular targeting of polymer-bound drugs for cancer chemotherapy. *Adv Drug Deliv Rev.* 2005; 57:609–636. [PubMed: 15722167]
77. Torchilin VP. Recent approaches to intracellular delivery of drugs and DNA and organelle targeting. *Annu Rev Biomed Eng.* 2006; 8:343–375. [PubMed: 16834560]

78. Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. *Nat Rev Clin Oncol*. 2010; 7:653–664. [PubMed: 20838415]
79. Gentile F, Ferrari M, Decuzzi P. The transport of nanoparticles in blood vessels: the effect of vessel permeability and blood rheology. *Ann Biomed Eng*. 2008; 36:254–261. [PubMed: 18172768]
80. Merkel TJ, Jones SW, Herlihy KP, Kersey FR, Shields AR, Napier M, Luft JC, Wu H, Zamboni WC, Wang AZ, Bear JE, DeSimone JM. Using mechanobiological mimicry of red blood cells to extend circulation times of hydrogel microparticles. *Proc Natl Acad Sci U S A*. 2011; 108:586–591. (S586/1–S586/6). [PubMed: 21220299]
81. Ramanujan S, Pluen A, McKee TD, Brown EB, Boucher Y, Jain RK. Diffusion and convection in collagen gels: implications for transport in the tumor interstitium. *Biophys J*. 2002; 83:1650–1660. [PubMed: 12202388]
82. Robinson KR, Messerli MA. Left/right, up/down: the role of endogenous electrical fields as directional signals in development, repair and invasion. *Bioessays*. 2003; 25:759–766. [PubMed: 12879446]
83. Glaser, R. *Biophysics*. Springer; New York: 2000. p. 171-176.
84. Arnott S, Fulmer A, Scott WE, Dea ICM, Moorhouse R, Rees DA. Agarose double helix and its function in agarose gel structure. *J Mol Biol*. 1974; 90:269–284. [PubMed: 4453017]
85. Serwer P, Hayes SJ. Exclusion of spheres by agarose gels during agarose gel electrophoresis: dependence on the sphere's radius and the gel's concentration. *Anal Biochem*. 1986; 158:72–78. [PubMed: 3799974]
86. Stellwagen NC. Agarose gel pore radii are not dependent on the casting buffer. *Electrophoresis*. 1992; 13:601–603. (Weinheim, Fed. Repub. Ger.). [PubMed: 1333950]
87. Johnson EM, Deen WM. Hydraulic permeability of agarose gels. *AIChE J*. 1996; 42:1220–1224.
88. Zanchet D, Micheel CM, Parak WJ, Gerion D, Alivisatos AP. Electrophoretic isolation of discrete au nanocrystal/DNA conjugates. *Nano Lett*. 2001; 1:32–35.
89. Hanauer M, Pierrat S, Zins I, Lotz A, Soennichsen C. Separation of nanoparticles by gel electrophoresis according to size and shape. *Nano Lett*. 2007; 7:2881–2885. [PubMed: 17718532]
90. Liu Y, Shipton MK, Ryan J, Kaufman ED, Franzen S, Feldheim DL. Synthesis, stability, and cellular internalization of gold nanoparticles containing mixed peptide-poly(ethylene glycol) monolayers. *Anal Chem*. 2007; 79:2221–2229. [PubMed: 17288407]
91. Perrault SD, Chan WCW. Synthesis and surface modification of highly mono-dispersed, spherical gold nanoparticles of 50–200 nm. *J Am Chem Soc*. 2009; 131:17042–17043. [PubMed: 19891442]
92. Hill RJ, Saville DA, Russel WB. Polarizability and complex conductivity of dilute suspensions of spherical colloidal particles with uncharged (neutral) coatings. *J Colloid Interface Sci*. 2003; 268:230–245. [PubMed: 14611793]
93. Hill RJ, Saville DA. 'Exact' solutions of the full electrokinetic model for soft spherical colloids: electrophoretic mobility. *Colloids Surf, A*. 2005; 267:31–49.
94. Hill RJ. Electric-field-induced force on a charged spherical colloid embedded in an electrolyte-saturated brinkman medium. *Phys Fluids*. 2006; 18:043103/1–043103/7.
95. Cheng Y, Meyers JD, Agnes RS, Doane TL, Kenney ME, Broome AM, Burda C, Basilion JP. Addressing brain tumors with targeted gold nanoparticles: a new gold standard for hydrophobic drug delivery? *Small*. 2011; 7:2301–2306.
96. Fourches D, Pu D, Tassa C, Weissleder R, Shaw SY, Mumper RJ, Tropsha A. Quantitative nanostructure–activity relationship modeling. *ACS Nano*. 2010; 4:5703–5712. [PubMed: 20857979]
97. Petros RA, De Simone JM. Strategies in the design of nanoparticles for therapeutic applications. *Nat Rev Drug Discov*. 2010; 9:615–627. [PubMed: 20616808]
98. Li M, Al-Jamal KT, Kostarelos K, Reineke J. Physiologically based pharmacokinetic modeling of nanoparticles. *ACS Nano*. 2010; 4:6303–6317. [PubMed: 20945925]
99. Dobson PD, Kell DB. Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule? *Nat Rev Drug Discov*. 2008; 7:205–220. [PubMed: 18309312]

100. Sugano K, Kansy M, Artursson P, Avdeef A, Bendels S, Di L, Ecker GF, Faller B, Fischer H, Gerebtzoff G, Lennernaes H, Senner F. Coexistence of passive and carrier-mediated processes in drug transport. *Nat Rev Drug Discov.* 2010; 9:597–614. [PubMed: 20671764]
101. Xue M, Zhong X, Shaposhnik Z, Qu Y, Tamanoi F, Duan X, Zink JI. pH-operated mechanized porous silicon nanoparticles. *J Am Chem Soc.* 2011; 133:8798–8801. [PubMed: 21595466]
102. Ferris DP, Lu J, Gothard C, Yanes R, Thomas CR, Olsen JC, Stoddart JF, Tamanoi F, Zink JI. Synthesis of biomolecule-modified mesoporous silica nanoparticles for targeted hydrophobic drug delivery to cancer cells. *Small.* 2011; 7:1816–1826. [PubMed: 21595023]

Fig. 1. Illustration of drug loading schemes relying on (A) surface mediated delivery and (B) encapsulation based techniques. The drug (green) is immobilized on the NP coated with a protective polymer corona (black) which can include further functionalities including targeting ligands or imaging agents (red).

Fig. 2. Examples of (A) surface mediated hydrophobic drug loading as reported by Kim et al. for zwitterionic NPs and (B) encapsulation of hydrophobic drugs as reported by Lu et al. in mesoporous silica NPs. Reprinted with permission from references [14] (A) and [13] (B). Copyright 2009 American Chemical Society and 2007 John Wiley and Sons, respectively.

Fig. 3.

Image of PEGylated Au NPs in solution (1) and mixed with a hydrophobic drug with [Drug:Au NP] concentration ratios of (2) 20:1, (3) 40:1, (4) 50:1, and (5) 60:1 in chloroform. Note that the red color indicates well dispersed particles, and the blue color indicates free drugs after NP aggregation.

Reprinted with permission from reference [5]. Copyright 2008 American Chemical Society.

Fig. 4. Calculated van der Waals attraction potentials for (A) gold nanoparticles of increasing size and (B) 5 nm nanoparticles of different materials as a function of distance. Hamaker constants for gold were used from ref. [33] and all others from ref. [31] according to Eq. (2) [29].

Fig. 5.

An example of the potential curve for 25 nm citrate coated Au NPs which include V_{vdW} and V_R . The curves change with time (numbers) after the addition of benzyl mercaptan due to substitution of citrate at the particle surface.

Reprinted with permission from reference [33]. Copyright 2005 American Chemical Society.

Fig. 6. Calculations of the ligand thickness as a function of PEG molecular weight for Au NPs with different surface curvature. Experimentally observed values are denoted as a red dashed line and the PEG contour length is denoted as a black dashed line. Insets show a Au NP with a constant 25 nm hydrodynamic radius but varying hard-to-soft volume ratios. Reprinted with permission from reference [20]. Copyright 2012 American Chemical Society.

Fig. 7. Illustration of the adsorption of proteins onto Au NP surfaces for both monodisperse particles and aggregates. Particles are initially stabilized by electrostatic repulsion (E_{ES}) to counter van der Waals attractive forces (E_{vdW}), but upon addition to biologically relevant serum are effectively coated with different proteins. Reprinted with permission from reference [44]. Copyright 2011 American Chemical Society.

Fig. 8.

Illustration of the complex environment NP-drug conjugates experience upon intravenous delivery. Particles initially flow through the vascular system (red), diffuse through the leaky endothelial walls associated with several diseases (dark grey), and finally reach the targeted cell (enlarged box).

Reprinted with permission from reference [12]. Copyright 2007 Nature Publishing Group.

Fig. 9.

Fluorescence imaging comparison of the migration of dye loaded silica NPs (red) and gel encapsulated QDs (green) in collagen gel (second harmonic generation, blue) both (A–C) before and (E–G) after addition of matrix metalloproteinases (MMPs). While the larger silica NPs ($d_h=100$ nm) are essentially trapped in the matrix, the QDs ($d_h=10$ nm) easily migrate after degradation of the encapsulating hydrogel.

Reprinted with permission from reference [62]. Copyright 2011 Proceedings of the National Academy of Sciences.

Fig. 10.

Fluorescence imaging comparison of the migration of gel encapsulated QDs (green) and dye loaded silica NPs (red) in the tumor matrix as a function of time (1–6 h). Note that while the silica NPs ($d_h=100$ nm) remain immobilized within the tumor vasculature, the gel surrounding the QDs is degraded and the smaller QDs ($d_h=10$ nm) are easily able to diffuse through the tumor, in agreement with the collagen gel model (Fig. 9). Reprinted with permission from reference [62].

Copyright 2011 Proceedings of the National Academy of Sciences.

Fig. 11.

Comparison of the biodistribution of (A) the noncovalently loaded drug and (B) the corresponding NP transporter (PEGylated Au NPs) at three different time points (4 h, 24 h, and 7 days) over the period of a week. While the uptake and clearance profile were shown to be similar, the distribution in other organs does not have a direct correlation, with the drug mainly residing in the tumor, stomach and large intestine and the particles within the spleen, kidneys, and urinary tract.

Reprinted with permission from reference [6]. Copyright 2011 American Chemical Society.

Fig. 12.

Electrophoresis of 6 nm Au NPs with varying PEG molecular weights (1, 2, 5, and 10 kDa) in 42 mM I.S. tris acetate EDTA (TAE) buffer at 80 V. (A) and (B) are identical experiments within the same gel to demonstrate reproducibility. Insets are a representative transmission electron microscopy (TEM) image of the NPs and UV-vis spectra indicating monodispersity.

Reprinted with permission from reference [48]. Copyright 2010 American Chemical Society.

Fig. 13.

Electrophoresis of ≈ 5 nm Au NPs coated with 80% 5 kDa HS-mPEG and (1) 10% COOH bifunctional PEG (2) 10% EGF peptide and (3) 10% EGF peptide and drug payload. Running parameters were 120 V for 4 h in 42 mM ionic strength TAE buffer. Reprinted with permission from reference [95]. Copyright 2011 John Wiley and Sons.

Fig. 14.

An example of hydrophobic drug delivery utilizing the encapsulation method. A general schematic (A) illustrates how mesoporous silica nanoparticles (MSNs) are effectively loaded with camptothecin and subsequently modified with targeting ligands. The resulting effect on a cancerous cell line (B) shows an approximate two and three fold increase in therapeutic efficiency for untargeted and targeted drug loaded MSNs in comparison to the free drug, with no significant cytotoxicity for the NPs themselves.

Reprinted with permission from reference [102]. Copyright 2011 John Wiley and Sons.