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Targeted polymeric therapeutic nanoparticles: design, development and clinical translation†

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Abstract

Polymeric materials have been used in a range of pharmaceutical and biotechnology products for more than 40 years. These materials have evolved from their earlier use as biodegradable products such as resorbable sutures, orthopaedic implants, macroscale and microscale drug delivery systems such as microparticles and wafers used as controlled drug release depots, to multifunctional nanoparticles (NPs) capable of targeting, and controlled release of therapeutic and diagnostic agents. These newer generations of targeted and controlled release polymeric NPs are now engineered to navigate the complex *in vivo* environment, and incorporate functionalities for achieving target specificity, control of drug concentration and exposure kinetics at the tissue, cell, and subcellular levels. Indeed this optimization of drug pharmacology as aided by careful design of multifunctional NPs can lead to improved drug safety and efficacy, and may be complimentary to drug enhancements that are traditionally achieved by medicinal chemistry. In this regard, polymeric NPs have the potential to result in a highly differentiated new class of therapeutics, distinct from the original active drugs used in their composition, and distinct from first generation NPs that largely facilitated drug formulation. A greater flexibility in the design of drug molecules themselves may also be facilitated following their incorporation into NPs, as drug properties (solubility, metabolism, plasma binding, biodistribution, target tissue accumulation) will no longer be constrained to the same extent by drug chemical composition, but also become in-part the function of the physicochemical properties of the NP. The combination of optimally designed drugs with optimally engineered polymeric NPs opens up the possibility of improved clinical outcomes that may not be achievable with the administration of drugs in their conventional form. In this *critical review*, we aim to provide insights into the design and development of targeted polymeric NPs and to highlight the challenges associated with the engineering of this novel class of therapeutics, including considerations of NP design optimization, development and biophysicochemical properties. Additionally, we highlight some recent examples from the literature, which demonstrate current trends and novel concepts in both the design and utility of targeted polymeric NPs (444 references).

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1. Introduction

The application of nanotechnology to developing safer and more effective medicines (nanomedicine) is set to substantially influence the landscape of both pharmaceutical and biotechnology industries for decades to come.[1–3] This increasing interest in nanomedicine is driven in large part by a fast pace of innovation and emerging successes of nanoparticle (NP) based drug delivery systems.[4] Discoveries in the field of nanomedicine have so far proven to be both evolutionary and revolutionary in nature.[5] The promotion of interdisciplinary research and the discovery of colloidal mechanisms of drug delivery in the 1960's and 1970's led to the development of the earlier nanomedicines; liposomes[6] and polymer-drug conjugates.[7, 8] The evolution of these NPs was followed by their successful "stealth" rendition by modifying the NP surface using polyethylene glycol (PEG) polymers in order to prevent non-specific binding of NP surfaces to blood components and reduce their rapid uptake and clearance *in vivo* by the cells of the mononuclear phagocytic system (MPS), leading to prolonged blood circulation times.[21] Following the development of antibody technologies came the ability to potentially increase NP specificity through bioconjugation of affinity ligands, such as antibodies, antibody fragments, peptides, aptamers (Apts), sugars, and small molecules to their surface in order to create targeted NPs.[12, 21–23] Fig. 1 presents a timeline for the development of several distinct NPs, which have been either approved for human use or are undergoing clinical trials including: liposome, albumin, and polymeric NPs. In addition to these, polymer coated iron oxide NPs have also been approved by the Food and Drug Administration (FDA) for use as magnetic resonance imaging (MRI) contrast agents.

Potential advantages of therapeutic NPs include: (1) the ability to improve the pharmaceutical and pharmacological properties of drugs, potentially without the need to alter drug molecules, (2) enhancement of therapeutic efficacy by targeted delivery of drugs in a tissue- or cell-specific manner, (3) delivery of drugs across a range of biological barriers including epithelial and endothelial, (4) delivery of drugs to intracellular sites of action, (5) the ability to deliver multiple types of therapeutics with potentially different physicochemical properties, (6) the ability to deliver a combination of imaging and therapeutic agents for real-time monitoring of therapeutic efficacy and, (7) possibilities to develop highly differentiated therapeutics protected by a unique set of intellectual properties.[7, 24]

With respect to NP research, targeting refers to differential spatial localization and describes the intentional homing of NPs to active sites in disease conditions and is distinct from molecularly targeted drugs. While molecularly targeted drugs preferentially modulate the function of proteins abnormally expressed or activated in a disease state, they are not designed for spatial localization and indiscriminately distribute within the body, contributing to off-target adverse effects.[25] This differential spatial localization of NPs encompasses two different approaches, which are "passive" or "active" targeting. Passive targeting refers to the preferential accumulation of NPs (bearing no affinity ligands) at active sites and is directly related to the inherent biophysicochemical properties of the NP (size, shape, charge and flexibility *etc.*). These biophysicochemical properties may also impede the effective concentration of NPs at active sites due to competitive events manifested by the MPS system leading to the sequestration of NPs, thereby limiting their systemic concentration and potential to extravasate into target tissues or bind to target cell populations.[26] Active targeting is a term used to describe the mode of action of NPs with surface modification to incorporate affinity ligands with specificity to disease tissues and cells. These NPs differentially bind to target molecules as a result of the binding properties of the ligands on the NP surface (passive and active targeting are further described in section 3). Although more than 30 years have gone by since the implementation of the concept of targeted NPs,

only a handful of these targeted NPs have reached clinical development and none have been clinically approved (Table 1).[29–31] Limiting factors such as: (1) insufficient understanding of events at the nano-bio interface both *in vitro* and *in vivo*, (2) inadequate knowledge of the fate of NPs at the body, organ, and cellular levels, (3) difficulty in achieving reproducible and controlled synthesis of NPs at scales suitable for clinical development and commercialization, and, (4) lack of technologies enabling screening of a large number of NP candidates under biologically relevant conditions that could be reliably correlated to clinical performance are some possible explanations for the slow clinical translation of these nanomedicines. Though a vast array ofmaterials have been used to formulate NPs for drug delivery, including polymers, lipids, carbon, silica oxides, metal oxides and semiconductor nanocrystals,[32] this review focuses specifically on targeted controlled release polymeric NPs for therapeutic applications. This focus stems from the potential impact of polymers on medicine as evidenced through previous clinical successes of polymers as biomedical materials,[2, 10, 14, 33] as well as their potential as targeted therapeutic NPs.[34]

1.1 Polymeric therapeutic nanoparticles: drug delivery vehicles or a novel class of therapeutics?

The first generation clinically approved NP drug delivery technologies (liposomes, micelles, proteins *etc.*) lacked controlled release and active targeting properties, and were able to generally improve the safety and efficacy of the active drugs they carried. Among these first generation NPs, DOXIL, Abraxane and Genexol-PM were developed for cancer therapy. DOXIL was the first FDA approved liposome nanomedicine to reach clinical approval in 1995 for AIDS related Kaposi's syndrome.[35] By encapsulating doxorubicin (Dox) within liposomes, DOXIL changed the pharmacokinetics (PK) and biodistribution (BD) of Dox, facilitating a longer circulation half-life and therefore higher tumour dose accumulation of this drug. Although the maximum tolerated dose (MTD) of DOXIL (50 mg m^{-2} every 4 weeks) was lower than that of standard Dox (60 mg m^{-2} every 3 weeks) and DOXIL exhibited a new toxicity of hand-foot syndrome (*palmar-plantar erythrodysesthesia*), however, in this case the therapeutic index of Dox was enhanced. This enhancement was due to the fact that the cardiotoxicity associated with the free drug (Dox) was reduced and efficacy was demonstrated in taxane/platinum-resistant ovarian cancers.[36] Despite the clinical validation of liposome technology, this class of NPs generally lack controlled release properties that can control the kinetics of drug exposure at the target tissue, and liposomes are also comparatively less stable as compared to polymeric NPs (discussed further in section 2).[37, 38] The approval of Abraxane (nab-paclitaxel) in 2005 by the FDA, which is based on the NP albumin-bound (nab) platform, led to the second class of therapeutic NPs to be clinically validated.[39] In comparison to paclitaxel (Ptxl) formulated with Cremophor EL (Taxol), Abraxane demonstrated significantly higher tumour response rates (33% *vs.* 19%) and longer times to tumour progression (23.0 *vs.* 16.9 weeks) among metastatic breast cancer patients who did not respond to combination therapy.[40] Upon administration of Abraxane, the NP formulation rapidly dissociates into its constituents of albumin and Ptxl molecules and therefore does not materially impact the circulation half-life or BD profile of Ptxl.[41] However, the nab platform significantly improved the MTD of Ptxl (260 mg m^{-2} *vs.* 175 mg m^{-2} for Taxol every 3 weeks) by removing the need for the use of the toxic excipient—Cremophor EL.[16, 42] Therefore, nab-technology does not dramatically improve drug PK or BD, and the utility of this technology is largely limited to improving the therapeutic index of hydrophobic drugs that are currently formulated with poorly tolerated solvents. Furthermore, not all validated drugs can bind to albumin which further limits the utility of the nab-technology. Genexol-PM (a Ptxl loaded polymeric micelle) was approved in Korea in 2007. This polymeric micelle technology also removed the need for the use of Cremophor EL leading to an increase of Ptxl MTD up to 300 mg m^{-2} every 3 weeks for

breast cancer treatment.[39] Genexol-PM is currently in phase II clinical development in the USA.[17, 43]

Each clinically validated NP platform has made sufficient improvement to drug safety and efficacy for successful approval, yet each platform has unique limitations, and most clinicians would argue that none have made a marked improvement in clinical outcomes. Today there are nearly 250 nanomedicine products in various stages of preclinical and clinical development.[44] For a NP platform to maximally improve drug pharmaceutical and pharmacological properties resulting in a highly differentiated new therapeutic with superior safety and efficacy, in most cases it will need to predictably change drug PK, BD, and tissue exposure kinetics-in a tunable and predictable manner. The successful development of such NP platforms is expected to create an entirely novel class of therapeutic NPs. The combination of one or more drugs with controlled release polymeric biomaterials for tuneable drug exposure, and molecular targeting for differential delivery has the potential to create novel therapeutic NPs for a range of medical applications.[18, 45, 46] These efforts may yield NPs with highly differentiated drug pharmacology and efficacy, analogous to creating novel drugs through conventional medicinal chemistry. Polymeric NPs have the capability to: (1) release drugs at an experimentally predetermined rate over a prolonged period of time, (2) release drugs preferentially at target sites with the possibility of controlled release rates, (3) maintain drug concentrations within therapeutically appropriate ranges in circulation and within tissues and; (4) protect drugs (small molecules, proteins, nucleic acids or peptides) from hepatic inactivation, enzymatic degradation and rapid clearance *in vivo*. Polymeric NPs encapsulate various drugs and release them in a regulated manner *via* diffusion of the drug molecules through the polymer matrix or *via* differential surface and bulk erosion rates of the particles. The systematic design of these systems allows for the fine-tuning and optimization of the exact polymeric NP composition that can lead to increased efficacy *in vivo*. By careful selection of the composition of polymeric NPs resulting in optimal PK/BD, the total amount of drug and the duration of drug exposure in target tissue can be altered and improved substantially. Additionally, the incorporation of targeting ligands on NPs can lead to their increased uptake and their active agents, leading to enhanced therapeutic outcomes. In this regard, targeted polymeric NPs have the potential to be highly differentiated therapeutics, distinct from the original active agents used in their composition and distinct from first generation NPs that largely facilitated drug formulation. Additionally, the higher intracellular concentration of drugs delivered by targeted polymeric NPs can potentially maximize therapeutic efficacy by overcoming drug resistance mediated by multidrug resistance (MDR) proteins.[47–49] The sub-cellular targeting of NPs can result in highly specific delivery of drug payloads to intracellular targets.[50] Several MDR transporters exist, of which the p-glycoprotein, human multidrug resistance protein (MRP1) and the breast cancer resistance protein have been widely studied.[51] Drug delivery using targeted polymeric NPs can result in continued release of drugs at a high concentration directly within the cell, potentially overcoming drug efflux.[49] Several studies in drug-resistant mouse models have demonstrated an enhanced antitumour activity for targeted NPs (*e.g.* folate-receptor targeted polymeric micelles, transferrin-conjugated Ptxl NPs *etc.*),[52–54] in comparison to their non-targeted equivalents which were shown to be not as effective. This review focuses on the development of targeted polymeric NPs and aims to highlight and discuss benefits and challenges of this novel class of therapeutics.

2. Controlled release polymeric NPs from discovery to the clinic

Controlled release systems generally refer to technologies or biomaterials that can be engineered to release drugs at predetermined and/or tuneable rates, or in response to external stimuli and triggers. Polymeric materials have emerged as a major class of controlled release systems since their unique physicochemical, synthetic, biocompatibility, and degradation

properties can be readily manipulated using well-established techniques.[55–57] Additionally, polymeric NP systems may be able to overcome the limitations of lipidic NPs such as liposomes. For example some key limitations of liposomes include: their propensity to burst release cargo *in vivo*, a lack of compatibility with various active agents, a limited drug loading volume, the oxidation of liposomal phospholipids, and poor shelf-life stability.[3, 18, 58] In contrast, polymeric drug delivery systems are comparably stable *in vivo*, have high drug loading capacities, and can employ both controlled or triggered release of drugs.[59] Due to these properties, polymeric nanomaterials are well positioned to continue to provide a diversity of solutions to a range of problems in medicine. In this section, we will provide an overview of selected developments that have led the way for the clinical translation of controlled release polymeric NPs.[60]

Polymers have been recognized for their potential in drug delivery applications since the 1960's.[61] However, at that time, many biomaterials were simply repurposed from industrial or household applications and therefore had inherent limitations. This began to change in the 1970's, particularly after seminal work by Langer and Folkman in 1976 which demonstrated that controlled release of macromolecules from biodegradable polymers in a temporal manner was possible.[10] A variety of macromolecules, which previously could not have been used as drugs due to PK or toxicity concerns could now be encapsulated into slow-releasing polymeric drug reservoirs and thereby developed into therapeutics suitable for use in humans. Over the past 4 decades controlled release polymer technology has impacted virtually every branch of medicine including ophthalmology, pulmonary, pain medicine, endocrinology, cardiology, orthopaedics, immunology, neurology and dentistry.[62] The annual worldwide market of controlled release polymer systems which extends beyond drug delivery is now estimated at $60 billion and these systems are used by over 100 million people each year.[62]

Today, the most commonly used polymers for controlled drug release applications include poly(D,L-lactide-*co*-glycolide) (PLGA), poly(lactic acid) (PLA), poly(glutamic acid) (PGA), poly(caprolactone) (PCL), *N*-(2-hydroxypropyl)-methacrylate copolymers (HPMA), and poly(amino acids).[63] In particular, PLGA, PGA and PLA (Fig. 2) have been widely used in an impressive number of controlled release products, particularly due to their favourable biocompatibility and biodegradability properties. These stem, in part, from simple clearance of the polymer matrix by the body's homeostatic metabolic pathways.[64]

Following the earlier work of Langer and Folkman, interest began to grow in developing slow releasing drug depots including surgical implants and injectable microparticles. The focus of these controlled release systems was principally to enable the potential use of macromolecules that had short half-lives as therapeutics, to enhance patient compliance, to improve drug efficacy and reduce side effects by delivering agents locally, and to simplify dosing in cases where prolonged drug exposure was necessary.[65] Years of preclinical work done in parallel and in collaboration by several groups in the 1970s, 1980s, and 1990s ultimately led to a number of clinical successes. For example, Zoladex, a PLGA copolymer impregnated with Goserelin acetate for treating breast and prostate cancers was approved by the FDA in 1998. It was designed to be injected subcutaneously so that the active agent could be released slowly into systemic circulation and reach its target sites.[66] The same year Lupron Depot, a PLGA microsphere formulation of leuprolide acetate, was approved by the FDA to treat advanced prostate cancers.[67] Among other notable controlled release formulations that followed is Gliadel, a biodegradable Polifeprosan 20 carmustine-embedded wafer for the treatment of gliomas that became the first new treatment for gliomas in 20 years on its approval in 1996,[68] and Atridox, a polylactide and *N*-methyl-2-pyrrolidone (NMP) polymer blend containing doxycycline hyclate for subgingival delivery, which was FDA approved in 1998 to treat periodontal disease.[69] Other controlled release

formulations of note include Sandostatin LAR, a PLGA slow release formulation of octreotide acetate for tumour control in neuroendocrine disorders approved in 1998.[70] Trelstar Depot, a PLGA based microparticle formulation of triptorelin pamoatea used for prostate cancer and other indications, was FDA approved in 2000.[71] The evolving ability to manufacture and control the assembly of polymers to nanoscale dimensions combined with growing interest in applying nanotechnology to medicine drove the downsizing of controlled release drug depots from macro or micro-scale products to the nano-scale.[72] Indeed the clinical success of these initial formulations both validated the concept of controlled release from polymers and set the stage for the coming of the polymeric NP era.

3. NP differential spatial localization; by passive or active means

3.1 Passive targeting

Currently, all of the clinically validated therapeutic and imaging NPs are considered passively targeted first generation nanomedicines. [7] The majority of these NPs exhibit prolonged circulation times *in vivo* and accumulate at particular sites simply due to blood hemodynamic forces and diffusive mechanisms. Passive targeting is widely exploited in oncology applications since, in particular, tumours facilitate accumulation of NPs through the widely reported “enhanced permeation and retention” (EPR) effect. This was a milestone discovery made by Maeda *et al.*, who in the 1980’s demonstrated the principle of passive targeting of colloidal particles to tumours.[73] In their initial studies, significantly higher concentrations of the cytotoxic drug neocarzinostatin was discovered in tumour tissue post administration of the polymer-drug conjugate poly(styrene*co*-maleic acid)-neocarzinostatin (SMANCS), in comparison to control experiments where the drug was administered in its free form.[73] This led Maeda *et al.* to postulate that the enhanced accumulation of the colloidal particles in the tumour was attributed to the structural features of the tumour vasculature, an observation, which was termed the EPR effect.[74] The EPR effect has been observed with a wide range of macromolecular agents such as proteins; including immunoglobulin G (IgG), drug-polymer conjugates, micelles, liposomes, polymeric NPs and many other types of NPs.[63, 75–77]

Tumour tissue is highly heterogeneous and is perfused by an aberrant and leaky microvasculature. Indeed, tumour microvasculature has been shown to be characterized by excessive branching, chaotic structures, enlarged inter-endothelial gaps with associated break-down of tight junctions between endothelial cells, and a disrupted basement membrane.[78] These large gaps between endothelial cells facilitate the extravasation of particulate material from the surrounding vessels into the tumour.[79] In addition to large leaky endothelial gaps, an impaired lymphatic drainage system further entraps macromolecular particles and delays their clearance. EPR is most effective for colloidal material of >40 kDa and can occur even in the absence of targeting ligands on NPs.[73] The size cut-off thresholds between endothelial cells varies between tumour type, though permeability and extravasation of NPs up to 400 nm through endothelial gaps has been observed (in mouse xenograft models of human cancers).[80] In addition to abnormal architecture, tumour blood vessels also have impaired receptors for angiotensin II which controls vessel constriction.[81] Solid tumours often produce large concentrations of vascular permeability factors as a result of rapidly growing tumour cells that require an increased supply of nutrients and oxygen. There are a number of vascular mediators which facilitate the EPR effect and these include; bradykinin, nitric oxide (NO), peroxynitrite ($ONOO^-$), prostaglandins, angiotensin-converting enzyme (ACE) inhibitors, vascular endothelial permeability factor (VEGF) and numerous other cytokines.[82] These factors are all indeed mediators of inflammatory processes and as such it is not surprising that the EPR effect may also manifest in other inflammatory scenarios such as arthritis, infection and advanced atherosclerotic plaques.[73, 83]

Currently the observations of EPR are the main premise for the design of tumour specific nanomedicines for drug delivery or imaging applications, however there are a number of caveats that need to be considered. For instance, the fact that large tumours show pathophysiological heterogeneity is a problem, as NPs cannot effectively accumulate throughout the tumour, in particular, the central regions of metastatic tumours do not exhibit the EPR effect which leads to lowered accumulation of colloidal NPs.[84] Furthermore, the degree of vascular permeability which ultimately leads to heterogeneity between tumour models and variable tumour microenvironments can affect the cut-off size for NP accumulation in tumours, restricting their effective penetration range, and additionally, also accounts for the lack of observable EPR effects in certain tumour types.[85, 86] Moreover, the negative pressure gradient present within the tumour interstitium can substantially limit the convection of NPs from the intravascular to the extravascular space within tumours, regardless of the presence of leaky vasculature.[85, 87] Since interstitial pressure is higher at the tumour core and diminishes outwards towards the tumour periphery rim, this can cause NPs to flow outwards from the tumour leading to a loss of effective drug dose within tumours. To circumvent these problems targeted NPs can be used for more efficient tumour or target tissue retention and cellular uptake, resulting in improved efficacy. Additionally, methods of elevating blood pressure or introducing NO-secreting compounds have been investigated by means of administering adjuvants in addition to NP injections.[82, 84] For example, VEGF can increase vascular permeability, and was shown to enhance the extravasation of NPs across tumour vasculature when co-administered with liposome NPs.[88] In addition to bradykinin, NO and prostaglandins that are factors involved in the regulation of vascular permeability, the administration of a number of kinase inhibitors has also led to an enhanced EPR effect.[89] The coadministration of a transforming growth factor beta (TGFβ) receptor inhibitor led to an enhancement of EPR mediated accumulation of both liposomal and micelle NPs, which was a direct result of reduction of pericyte coverage on tumour neovasculature.[89] By enhancing vascular permeability and lowering the pressure difference by raising blood pressure, the overall “leakiness” of tumour vessels and therefore passive accumulation of NPs can be increased.

The majority of passively targeted NPs possess a surface coated with PEG polymer for biocompatibility; however, this highly hydrophilic surface does not result in optimal endocytic uptake by cancer cells within the tumour. This problem which has been referred to by some as the “PEG dilemma”[90, 91] has been suggested to hamper efficient drug delivery in tumours as passively targeted NPs end up releasing their therapeutic payload into the tumour milieu rather than within cancer cells. However, in the case of cytotoxic drugs—many have been shown to have longer elimination half-lives in tumours *vs.* normal tissue. Therefore, the delivery of higher amounts of drugs to tumours can lead to longer durations of drug exposure at higher concentrations and enhanced efficacy.[92–95] For example, docetaxel (Dtxl) has an elimination half-life of 2.2–4.5 h in normal tissue and 22 h in tumours, demonstrating long tumour site retention relative to non-tumoural tissues.[96] For drugs that are not readily retained in tumours or macromolecular drugs that are not readily taken up by cancer cells, then extracellular drug release may be less effective at maintaining a differentially high tumour drug concentration over an extended period of time. This problem is further compounded with NP systems that lack controlled drug release properties. For example, micelle NPs can demonstrate a very rapid “burst” release post administration (releasing up to 50% of their encapsulated drug within 30 min) leading to premature drug release prior to effective EPR mediated tumour accumulation.[82] Similar problems exist for liposome based NPs, which can lead to either very slow or fast release of their therapeutic content. Furthermore, the administration of PEGylated liposomes has led to the production of PEG-specific antibodies,[97] causing the rapid clearance of a further administered dose—leading to an accelerated blood clearance (ABC) phenomena—which further diminishes

effective drug concentrations at tumour sites, but can be rectified by careful tuning of dose (discussed in more detail in section 3.2).[98]

Extensive efforts in forming PEGylated block copolymers have ultimately resulted in the clinical translation of a number of passively targeted polymeric NPs including; SP1049C,[99] NK911,[100] Genexol-PM and others, which are now in early phase clinical trials for treating a variety of cancers.[17] In general, these NPs are PEGylated polymeric micelle formulations. Polymer micelles are polymeric NPs that form from the self assembly of amphiphilic polymers at concentrations above the critical micelle concentration (CMC), yielding NPs which can encapsulate poorly water soluble drugs.[101] SP1049C is a pluronic polymeric micelle NP that is composed of a Dox-entrapping hydrophobic core and a hydrophilic polymer, and is currently undergoing phase II studies in patients with metastatic cancer of the esophagus and esophageal junction that have been refractive to standard chemotherapy treatments.[99] SP1049C was observed to be effective in bypassing p-glycoprotein-mediated drug resistance.[102] In this study, patients were treated with a single dose of SP1049C, 75 mg m^{-2} (Dox) given as an intravenous infusion every 3 weeks.[99] The results of this study and preclinical studies demonstrated superior anti-tumour efficacy for SP1049C when compared to free Dox administration.[99]

Two other passively targeted polymeric NPs are NK911, a micellar NP comprising PEG, Dox and poly(aspartic acid), and Genexol-PM, which is a Ptxl-encapsulated PEG-PLA micelle formulation currently in phase II development for various cancers.[17, 43, 103] As mentioned previously, Genexol-PM does not require the use of Cremphor EL, and has therefore led to an increase in Ptxl MTD for breast cancer therapy.[42, 104] Additionally, Genexol-PM administration demonstrated increased treatment response rates when given to patients who were not responsive to standard taxane therapy with Ptxl/carboplatin therapies, further suggesting improved outcomes for MDR cases. Xyotax (Ptxl-poliglumex), also a passively targeted polymeric NP in which Ptxl is conjugated to poly(L-glutamic acid), was shown to preferentially target ovarian tumours.[105, 106] Another example of a passively targeted polymeric NP undergoing phase trials is IT-101, a camptothecin-cyclodextrin polymer conjugate that has shown prolonged circulation times and slow drug release kinetics *in vivo*, both in pre-clinical and clinical studies.[107] These first generation polymeric NPs have so far demonstrated activity against tumours that have been resistant to standard therapies, and show promise in stabilizing disease in patients. The containment of drugs within these NPs leads to significantly reduced off target effects, which can lead to wider therapeutic windows and lower systemic toxicities. Passive targeting strategies are not without limitations and therefore considerable efforts are now underway to investigate actively targeted NPs that can further retain NPs at active sites. Currently there are three targeted polymeric NPs undergoing clinical trials which include: BIND-014, CALAA-01 and SEL-068; these targeted clinical stage NPs will be discussed further in section 3.3.

3.1.1 Long circulating polymeric NPs—Following the discovery of themany inherent advantages for the use of polymericmaterials in drug delivery applications, a landmark paper by Langer and colleagues in 1994 demonstrated that forming diblock copolymers of controlled release polymers with PEG could dramatically increase the circulation half-lives of polymeric NPs.[14] Since then, there have been a myriad of PEGylated polymericNPs reported in the literature with the benefits of PEGylation demonstrated across a broad range of polymer molecular architectures and macromolecular assemblies.[108–110] In addition to this extensive preclinical work, PEG has been validated clinically in many different applications, and is currently listed as “Generally Recognized as Safe” (GRAS) by the FDA, making it particularly attractive to translational researchers.[109] The success of PEG in transforming polymeric NP drug delivery has not been without its challenges however, some of which remain ongoing areas of investigation. For example, the induction of the

aforementioned ABC phenomena by PEGylated liposomes has been shown to be influenced by NP size, surface charge, constituents, and time period prior to second dose, and has been observed with other types of NPs, and even been shown to be dependent on NP therapeutic load and type.[111–114] However, observations of ABC phenomena have been conflicting in the literature so far as the induction of PEG specific antibodies have been observed in some cases and not in others—therefore given the variable design and composition of NPs, these effects should be investigated on a case-by-case basis.[112, 115, 116] A number of studies have now demonstrated that PEG appears to activate complement in a concentration and molecular weight dependent manner, through classical (C1q dependent), lectin, or alternative pathways.[117] While PEG is capable of both activating complement and eliciting anti-PEG antibody responses, the manner and extent of these immune responses can be modulated. Further, it has been shown that modifying the density of PEG on a NP surface alters the complement activation pathway, perhaps by altering the conformation of the PEG chains on the NP surface.[118] These data suggest that surface optimization of PEG density and molecular weight will be critical to avoid unwanted immune (non-IgE) hypersensitivity reactions. In addition to complement activation, as mentioned previously, PEGylated liposomes have been shown to elicit immunoglobulin M (IgM) antibodies in a number of reports, leading to ABC phenomena post-repeat dosing in a short time interval following initial dose administration.[98] Production of short term anti-PEG IgM appears to be dependent on the species tested, dose, PEG density, NP charge, and type of drug encapsulated. It is noted, that to attenuate the anti-PEG immune response, it appears important to tune the dose, shorten the PEG molecular weight, increase the density of PEG on the NP surface, tune NP surface charge close to neutral, and/or encapsulate an agent that attenuates macrophage function, such as Dox.[98] The clinical significance of these reports in terms of affecting PEGylated NP drug carriers has not been critically evaluated, though further preclinical and clinical data (Table 1) will at least provide an interim answer. At this point, with known methods to attenuate the anti-PEG immune response, and the well-established clinical success of the DOXIL (PEGylated liposome) and many other clinically validated PEGylated proteins, one can conclude that the anti-PEG immune response is not an intractable issue for polymeric NP carriers. Furthermore, alternatives to PEG are currently being developed, including new polymers or zwitterionic surfaces that are ultra-low fouling in nature.[119] The preclinical data for these systems is encouraging, and their further development and study in the context of NP drug delivery is widely anticipated.[109, 120, 121]

In order to achieve effective EPR mediated targeting, NPs must have long-circulating half-lives that facilitate more opportunities for the passage of NPs from the systemic circulation into the disordered and permeable regions of tumour vasculature. As mentioned previously, passive targeting strategies are not without limitations and therefore considerable efforts are now underway to investigate actively targeted NPs that can further retain NPs at active sites. Targeted NPs facilitate receptor-mediated endocytosis (RME), releasing therapeutic agents in a more effective manner once inside target cell populations,[122, 123] which can significantly increase drug efficacy.[51, 124, 125]

3.2 Active targeting

Active targeting involves the use of affinity ligands to direct the binding of NPs to antigens, differentially overexpressed on the plasma membrane of diseased cells or to the extra-cellular matrix proteins that are differentially overexpressed in the disease tissue. The first reports of targeted NPs date back to 1980's and involved the surface modification of liposomes with monoclonal antibodies (mAbs) that recognized antigens on the target cells.[22, 23, 126] There are 30 mAbs approved for clinical use to date.[127] Muromonab-CD3 (OKT3, immunosuppressive agent) was the first antibody to be approved in 1986.[128] Since

then a myriad of antibody platforms have been developed including murine, chimeric, humanized and human mAbs.[51] For example, the chimeric mAb rituximab (Rituxan), which binds to the CD20 antigen, was approved for the treatment of non-Hodgkin's lymphoma in 1997.[129] The humanized mAb trastuzumab (Herceptin) which binds to the HER2/neu antigen was approved for the treatment of breast cancer in 1998.[130] Stemming from the success of mAbs, several other classes of binding ligands were developed against many target antigens, including antibody mimetics, peptides, nucleic acid ligands and small molecules (see section 5). Many of these ligands have been conjugated to radioisotopes or drug molecules to create more effective targeted imaging and therapeutic modalities.[131–133] Subsequently, many of these ligands were also conjugated to the surface of NPs in order to achieve antigen-specific active targeting.[134] In contrast to ligand-drug conjugates which typically carry 1–8 drug molecules, ligand targeted NPs may carry up to 10^3 to 10^4 drug molecules, allowing for potentially a higher amount of drug delivery per bio-recognition or binding event.

Actively targeted NPs can be utilized in applications where drug release is either extracellular or intracellular. Therapies that act on intracellular sites of action are most effectively delivered with targeted NPs.[3, 135] Actively targeted NPs may be internalized *via* clathrin-dependent endocytosis pathways, caveolin-assisted, cell adhesion molecule directed, or lipid raft associated mechanisms, leading to endosome formation, which ultimately leads to lysosomes.[136] For hydrophobic small molecule drugs that can readily permeate through the lipid bilayer of the endosomal membrane, drug release within the endosome will result in permeation within the intracellular compartments. For delivery of bioactive macromolecules such as nucleic acids (DNA, siRNA, miRNA) or charged hydrophilic small molecules that are relatively impermeable to the endosomal membrane, the NPs need to escape the endosome prior to fusion with lysosomes if NPs are to reach their desired subcellular compartments.[137] Many efforts have led to the investigation of mechanisms that lead to endosomal escape based on pH buffering, osmotic swelling leading to endosome bursting or endosomal membrane destabilization.[138, 139] Ligand mediated cell internalization can result in enhanced therapeutic benefits as compared to equivalent non-targeted NPs.[124, 140] Experiments comparing targeted and non-targeted NPs have confirmed that the primary role of the targeting ligand is to enhance cellular uptake into target cells.[141, 142] For example, accumulation of siRNA-loaded NPs at tumour sites is largely a function of effective EPR *via* passive targeting; however, cellular internalization and effective gene silencing are largely a function of targeting ligand where targetedNPs are significantly more efficacious as compared to equivalent non-targeted NPs.[143, 144] This behaviour suggests that the colloidal properties of NPs determine their biodistribution, whereas the targeting ligand serves to facilitate and enhance cellular uptake at targeted sites.[145]

Ligand mediated targeting is also beneficial in the case of vascular endothelial targeting for both oncology and cardiovascular applications, and the identification of high affinity ligands for this purpose is an active area of research.[146] Recent studies have shown that small peptide targeted polymeric NPs showed substantial accumulation to injured vasculature following angioplasty compared to non-targeted NPs.[147] Interest in the use of short peptides as targeting ligands has increased. In comparison to larger mAb's peptide ligands have the advantage of being (i) smaller in size, (ii) less immunogenic, (iii) more stable; and (iv) easier to manufacture.[148] Peptides however, have relatively lower affinity for their target site, and this deficiency is in-part mitigated through ligand avidity which is achieved by incorporating multiple peptides on the NP surface.[149] The establishment of a wide range of phage display libraries and screening technologies has resulted in isolation of peptide ligands against many important targets (targeting ligands are discussed further in section 5).[150–152]

While the potential benefit of ligand-mediated NP targeting is clear, this technology has not resulted in a clinically validated product so far. Within the 32 years since the first description of targeted NPs, only six targeted NPs have progressed to clinical trials (Table 1). From these six NPs, three are targeted polymeric NPs and three are targeted liposomes. MCC-465 was the first of these to be developed and consists of liposome encapsulated Dox, with a surface decorated with both PEG and dimers of antigen-binding fragments ($F(ab')_2$) for immune shielding and targeting respectively.[213] The $F(ab')_2$ used in the development of this NP is a fragment of the human mAb, GAH which has shown affinity to >90% of human stomach cancer cells.[213] Additionally, antibody fragments may be preferred for certain applications since they retain the high affinity and specificity of antibodies but are smaller in size and therefore potentially less immunogenic.[51] MCC-456 was shown to exhibit significant antitumour response against GAH-positive xenografts resulting in up to 80% reduction in tumour mass in comparison to controls.[214] Phase I trials with MCC-465 were carried out in order to determine the MTD and further dosing regimens for Phase II analysis. In this study patients with metastatic cancer or recurrent stomach cancer were administered 6.5 mg m^{-2} of MCC-465 as a 1 h infusion every 3 weeks for up to 6 treatment cycles. It was concluded that MCC-465 was well tolerated and similar pharmacokinetic outcomes were observed as compared to DOXIL. However, MCC-465 does not appear to have progressed through clinical development after phase I completion.

SGT53-01 is a transferrin receptor (TfR)-targeted liposome designed to carry the p53 tumour suppressor gene to cancer cells.[215] SGT53-01 targets the TfR on the surface of cancer cells using single-chain antibody fragments (TfRscFv) and results in the expression of p53 gene in the targeted cancer cells. Pre-clinical studies have indicated that SGT53-01 could sensitize tumours to the effects of radiation and chemotherapy.[215] SGT53-01 is currently undergoing phase I clinical trials in combination with Dox for treatment of solid tumours.

MBP-426 is also a TfR-targeted liposome that encapsulates oxaliplatin and is designed to preferentially target the delivery of oxaliplatin to cancer cells.[216] Transferrin (Tf) is widely used as a targeting ligand since the TfR is significantly upregulated on most cancer cells.[217] In a phase I study, patients with advanced or metastatic solid tumours refractory to conventional therapy received MBP-426 as 2–4 h infusions every 3 weeks in cohorts of 3 to 6 patients, and this targeted liposome was demonstrated to be well tolerated (with thrombocytopenia as the main dose limiting toxicity (DLT)).[216]

The last decade has seen a variety of strategies involving conjugation of targeting ligands to the surface of NPs in order to provide molecular interaction points between the NPs and antigens present on target cells and tissues. What has emerged from these studies is that a variety of different targeting ligands can trigger NP internalization into cells, and that internalization can significantly enhance treatment efficacy.[51, 62] Table 2 highlights from the literature the wide range of targeted polymeric NPs along with their available physicochemical properties developed for numerous therapeutic applications.

3.3 Clinical stage targeted polymeric NPs

Conventional methods of preparing targeted NPs involve a series of chemical processes whereby the NP core is initially formed, followed by the bioconjugation of targeting ligands to the surface of the NP. This post-coupling of targeting ligands does not allow tuning of ligand density for optimal efficacy, requires excess amounts of reagent in order to achieve high coupling efficiencies, and is associated with purification techniques to remove unbound ligands. Due to this kind of complexity in the synthesis of NPs, difficulties may arise in the reproducibility of NP surface properties, resulting in batch-to-batch variability, which is not amenable to clinical translation and subsequent commercialization. Indeed, by reducing the

number of components to the minimum, and employing a modular self-assembly approach using pre-functionalized polymeric materials,[218] it is possible to create libraries of targeted NPs that vary narrowly from each other in their biophysicochemical properties. Using this strategy, BIND Biosciences recently developed and screened a library of targeted self-assembled polymeric NPs resulting in the development of BIND-014, the first targeted and controlled release polymeric NP for cancer chemotherapy to reach clinical development.[219] BIND-014 is a prostate specific membrane antigen (PSMA)-targeted Dtxl-encapsulated polymeric NP, which entered phase I clinical trials in January 2011.[219] PSMA is a transmembrane protein overexpressed on the surface of prostate cancer cells and tumour-associated neovasculature of virtually all solid tumours.[220, 221] Dtxl is a semi-synthetic taxane approved for treatment of a number of major solid tumour cancers, including breast, prostate, lung, gastric, and head and neck.[222] BIND-014 has been shown to deliver up to 10 times more Dtxl to tumours relative to an equivalent dose of Dtxl in multiple animal models.[219] Initial clinical data in patients with advanced solid tumours indicate that BIND-014 displays a pharmacological profile differentiated from Dtxl, including pharmacokinetic properties consistent with long circulation half-life of BIND-014 and retention of Dtxl in the vascular compartments, and multiple cases of tumour shrinkage at doses up to 5 times below the Dtxl dose typically administered clinically.[219]

CALAA-01 is the first targeted NP to reach clinical development for siRNA delivery in 2008.[18] The CALAA-01 NP consists of siRNA to reduce the expression of the M2 subunit of ribonucleotide reductase (R2), cyclodextrin containing polymer (CDP) for siRNA condensation, adamantine-PEG (AD-PEG) for steric stabilization, and adamantine-PEG conjugated to human Tf (AD-PEG-Tf) to target the TfR overexpressed on the surface of most cancer cells.[223] CALAA-01 employs a unique two-vial formulation strategy, which allows for the rapid self-assembly of the NP (50–70 nm) delivery system components (CDP, AD-PEG, AD-PEG-Tf) with siRNA, at the point of care (Fig. 3).[18] This formulation is also capable of high siRNA payload delivery and endosomal pH (<6.0) triggered release of siRNA once NPs are endocytosed.[224]

SEL-068 is a first-in-class synthetic and integrative targeted polymeric NP vaccine to reach phase I clinical development in November 2011. SEL-068 contains nicotine as antigen, T-helper cell peptides, TLR agonists as adjuvant, and is currently under development for smoking cessation and relapse prevention.[27] Post smoking, nicotine usually enters the lung and the systemic circulation and reaches the brain by crossing the blood-brain barrier (BBB), and binds to nicotine receptors resulting in release of stimulants such as dopamine leading to reinforcement of addiction.[225] The administration of SEL-068, which is based on modular self-assembly NP technology,[196] results in high anti-nicotine antibody concentrations and a high anti-nicotine antibody affinity; leading to the sequestration of nicotine molecules in circulation and largely blocking central nervous system exposure, thereby diminishing the addictive effects of nicotine.

The clinical translation of the above technologies has marked a new era in the development of multi-functional therapeutic NPs capable of targeting, controlled release, and co-delivery of multiple active agents.

4. Preparation of targeted polymeric NPs

4.1 Methods for preparing polymeric NPs

A number of top-downmethods are available for the preparation of polymeric NPs using biodegradable polymers. Most of these involve self-assembly of block copolymers that are composed of two distinct polymer chains with different solubilities. These methods include nanoprecipitation (also called solvent displacement method),[226] various types of

emulsification/solvent evaporation,[227] and the salting out method.[228] In addition to these conventional methods, new approaches used to create polymeric NPs, including supercritical technology, electro-spraying, premix membrane emulsification and aerosol flow reactor methods are also under investigation which are further discussed in a recent review.[229] The choice of NP formulation method is usually dependent on the drug physicochemical properties along with the requirements for encapsulation and particle size. Nanoprecipitation, oil-in-water (O/W) emulsification-solvent evaporation (single emulsion), and water-in-oil-in-water (W/O/W) emulsification-solvent evaporation (double emulsion), are three of the most commonly utilized methods to prepare a variety of polymeric NPs and will be described in the next paragraphs.

Nanoprecipitation is a method that involves the use of an organic solvent that is miscible with an aqueous phase.[230] In this technique, the polymer and drug are dissolved in the organic solvent and this solution is then added dropwise to an aqueous (non-solvent) solution under stirring. Once in contact with water, the hydrophobic polymers and drug precipitate and self-assemble into core-shell like spherical structures in order to reduce the system's free energy.[45] After self-assembly, the organic solvent is evaporated either by reduced-pressure evaporation, or simply by continuous mixing at atmospheric pressure if the solvent is relatively volatile. The instantaneous formation of particles is governed by the principles of the Marangoni effect and has been attributed to interfacial interactions between liquid phases.[231]

The use of diblock hydrophobic-PEGylated polymers in nanoprecipitation leads to NPs that consist of a hydrophobic core, with entrapped hydrophobic drugs, surrounded by a hydrophilic shell for steric stabilization.[232] For example, Dtxl-loaded NPs were prepared using nanoprecipitation, whereby the hydrophobic drug Dtxl was mixed and co-precipitated with the diblock polymer poly(lactic-*co*-glycolic acid)-polyethylene glycol-carboxylic acid (PLGA-PEG-COOH).[233] The resulting NPs had a hydrophobic core composed of PLGA, wherein Dtxl was encapsulated, and a hydrophilic shell composed of PEG. Nanoprecipitation is a simple method, amenable to scale-up at an industrial scale requiring only mild stirring under minimal shear stress. In general, smaller NPs are obtained through this method when compared to other methods at equivalent conditions. In contrast, some drawbacks include the poor entrapment of hydrophilic drugs (hydrophilic drugs can remain in the aqueous phase),[229] lower entrapment efficiencies compared to other methods and difficulty in complete removal of the organic solvent after self-assembly.[232] Although recent advances whereby the aqueous water phase (non-solvent) is replaced with other organic solvents (methanol, ethanol) has facilitated the use of this technique for the use of hydrophilic drugs also.[229]

Emulsification techniques (water-in oil: W/O, oil-in water: O/W and double emulsion: W/O/W) require the formation of emulsion, followed by the homogenization of this mixture, and although originally used to formulate microparticles, can now also be utilized to prepare nano-emulsions.[234] The type of emulsification method utilized ultimately depends upon the properties of the polymer, drug and also the degree of miscibility of the organic (oil) solvent with the water phase.

The single emulsion technique (O/W) requires the drug to be soluble in a water-immiscible organic solvent. In this method, the polymer and the drug are dissolved in a volatile water-immiscible solvent such as dichloromethane or ethyl acetate, and the organic phase is emulsified under intense shear stress into an aqueous phase containing appropriate amounts of a surfactant, such as sodium cholate or polyvinyl alcohol (PVA). The organic solvent is allowed to evaporate, allowing the self-assembly of NPs.[235] The O/W emulsification technique is suitable for entrapping hydrophobic drugs and generally results in higher drug

loading and encapsulation efficiency compared to nanoprecipitation, as well as achieving complete solvent removal. However it requires an additional input of energy such as sonication or homogenization and the resulting NPs are often larger than those obtained through nanoprecipitation.[235]

Double emulsion (W/O/W) is generally used for encapsulation of hydrophilic drugs and proteins. In this method the drug is dissolved in a small volume of an aqueous phase together with a surfactant and this is emulsified in an organic phase containing the polymer. The W/O emulsion formed is then dispersed in a larger volume of an aqueous phase with or without surfactant to form the double W/O/W emulsion. Finally, the solution undergoes evaporation of the remaining organic solvent yielding NPs.[235] This method normally yields NPs with larger size than nanoprecipitation or O/W methods, with moderate drug loading and encapsulation efficiency.[232]

In the aforementioned NP preparation methods, several factors affect the physicochemical properties of the NPs, such as the solvent of choice, the solubility of the drugs (*e.g.* log $P_{o/w}$), the mixing time of the aqueous and organic solvents, the type of surfactant used, the concentration of polymer in the organic solution, the ratio of organic to aqueous solution in addition to others.[45, 229, 232] For instance, Cheng *et al.*[191] systematically studied the effect of some of these factors on the physicochemical properties of PLGA-PEG NPs. Their data suggested that an increase in the water miscibility of the solvent led to a decrease in mean NP size, and a linear relationship between polymer concentration and size was further shown. The solvent/water ratio, however, did not have a clear relationship with NP size. In addition, the effect of drug loading on resulting NP size distributions was also investigated.[191] While plenty of examples exist that demonstrate the reproducible production of polymeric NPs using the range of aforementioned therapeutic NP preparation techniques—the ultimate challenge arises with the translation of these methods to industrial scale production levels. Despite the revolutionary developments in nanotechnology and the clinical translation of a range of NPs containing active pharmaceutical ingredients (APIs), greater strides are needed in NP synthesis, processing, scale-up and manufacturing.[229]

4.2 Microfluidic methods

Microfluidics, the science and technology of manipulating nanoliter volumes in microscale fluidic channels, has shown that several labour-intensive and time-consuming steps such as sample preparation, mixing, reactions, purification, separations, and detection could be performed on a single monolithic microfabricated device.[238] In the last few years, applications of microfluidics have expanded from conventional chemical and biological analysis to other fields such as chemical reactions, biochemical assays, and cell handling.[239] Two particularly important contributions have been the development of soft lithography in PDMS as a method for fabricating prototype devices, and the simple fabrication of pneumatically activated valves, rapid mixers and pumps on the basis of soft-lithographic procedures. This has resulted in the fabrication of prototype devices to test new ideas in approximately 2 days (from design to working device), whereas the same applications for silicon technology may take a month ormore for non-specialists to carry out.With respect to nanoparticles, the ability of microfluidic systems to mix reagents rapidly, provide homogenous reaction environments, continuously vary reaction conditions, enable rapid temperature control, and allow addition of reagents at precise time intervals—are some of the key features that have made microfluidic systems useful for the synthesis of NPs.[240] Furthermore, synthesis carried out in microchannels allows for in-line characterization,[241] feedback control,[242] and high-throughput continuous synthesis,[243] which potentially enables screening and optimization of libraries of nanoparticles with different properties.

Recently, technologies developed for the synthesis of polymeric NPs have demonstrated the tremendous potential for microfluidics to dramatically improve on current bulk synthesis methods. Polymeric NPs prepared by bulk synthesis tend to have variable physicochemical properties (size, surface composition, and drug loading) due to the inability to control the mixing of precursors.[236] Further post-processing by extrusion, freeze–thaw, sonication, and/or high-pressure homogenization is often required. Using rapid mixing techniques in micro-channels such as hydrodynamic flow focusing, polymeric NPs exhibiting narrow size distributions compared to bulk synthesis have been prepared in a reproducible manner (Fig. 4).[236, 237] In these systems size can be tuned by either varying the mixing time of precursors, which is achieved by varying the flow ratio of the precursor streams, by varying the molecular weight of the polymer, or by simply varying the concentration of the polymer in the organic solution. Remarkably, for polymeric NPs prepared through microfluidics, higher drug encapsulation without increase in NP size has been observed,[236] which is highly desired for therapeutic NPs. Another method to prepare NPs takes advantage of the rapid mixing microenvironment that occurs in micro-droplets formed inside microfluidic channels.[244] For instance, cross-linked alginate NPs were synthesized in a micro-channel using aqueous alginate droplets as templates, followed by the shrinkage of the drops. This method exhibited remarkable control over the NP properties, specifically size and size distribution. These are just a few examples showing the advantages of microfluidics for nanoparticle synthesis, and recent reviews discuss these concepts further.[240, 245] Given the volume of research currently involving the microfluidic synthesis of NPs, it is expected that as more therapeutic NPs reach a clinical stage, the need for improved synthesis methods would also increase, at which point microfluidic technologies could likely become an important tool in their development of NPs.

4.3 Drug loading methods

In general, drug loading into polymeric NPs can be achieved according to three techniques: (1) the drug is covalently attached to the polymer backbone, (2) the drug is adsorbed to the polymer surface, or, (3) the drug is entrapped in the polymer matrix during preparation of the NP.[246] In turn, drug release rates from NPs depend upon a number of parameters including: (a) diffusion through the NP matrix, (b) erosion of the NP surface and, (c) polymer matrix degradation (see Fig. 5).[51] In the following paragraphs we will discuss drug encapsulation by entrapping the drug during preparation and conjugation of the drug to the polymer backbone. Entrapping a drug during NP preparation is the most common technique used for incorporating drugs into NPs. For hydrophobic drugs the fact that they simply need to be mixed with the polymer in an organic solvent, makes it very attractive for formulation purposes. In fact, most NP formulations that are at the clinical stage rely on this method of encapsulation. However, this method suffers from some disadvantages mainly that of relatively low drug entrapment accompanied with low encapsulation efficiency at high loadings.[247] In addition, maximum encapsulation efficiencies tend to vary with drug type, and are also affected by the type of polymer, solvents, temperature, and mixing time of NP precursors among other factors.[248] Moreover, the encapsulation of two or more drugs with this method may be difficult to achieve, especially for drugs with different chemical properties. Conjugating drugs to the polymer backbone is an attractive alternative that minimizes some of the disadvantages encountered for drug entrapment.[249–252] This method involves chemically modifying the polymer (or the drug) to allow for chemical conjugation of the drug to the polymer. Originally, this method was accomplished by conjugating drugs to a functionalized end of a polymer chain. For instance, Sengupta *et al.* conjugated Dox, to PLGA, and were able to reproducibly prepare Dox-loaded NPs.[188] Similarly, Tong *et al.* developed a method to prepare Ptxl-conjugated PLA by carrying out a ring opening polymerization of LA units onto a Ptxl-metal complex.[253] This drug-conjugated polymer resulted in the reproducible synthesis of NPs incorporating high loadings of Ptxl with

encapsulation efficiencies close to 100%.[253] While the last two examples involved conjugation of drug molecules at the distal end of a polymer chain, recently Kolishetti *et al.* demonstrated both the encapsulation of Dtxl and the conjugation of a cisplatin pro-drug to formulate polymeric NPs for combination therapy.[254] This resulted in an increase in encapsulation together with a potential of tuning drug release kinetics by varying the number of drugs attached to the polymer.

In order to achieve optimal polymer-drug conjugate NP systems, the following considerations should be noted: (i) the chemistry implemented in modifying the drug should not affect the chemical groups or moieties that endow the drug with therapeutic effects; (ii) the drug needs to be cleavable from the polymer backbone under biological conditions, and upon cleavage the drug should retain its functional and therapeutic properties; (iii) chemistries used for conjugation must be carefully chosen since some residues of, for instance, certain catalysts, might contaminate the resulting NPs leading to unexpected toxicity. Nevertheless, conjugation of drugs to polymer backbones is an attractive strategy to incorporate multiple drugs with varying physicochemical properties in the same NP, enabling integrated and controlled combination chemotherapy.

4.4 Incorporation of targeting ligands on NPs

The widespread interest in the surface attachment of targeting ligands to various drug delivery NPs ranging from liposomes, micelles, polymers and dendrimers has spurred chemists to actively research coupling methods that are safe, tuneable, biocompatible, and reproducible. However, unlike the limited range of chemistries available for coupling to protein surfaces, a larger number of chemical modifications are now possible in order to attach targeting ligands to NPs. This can be done through either covalent attachment of targeting ligands to the surface of the NP or through electrostatic, dative or coordinate bonds. It is important to identify the conjugation technique that will ultimately provide the most efficient coupling chemistry that does not lead to undesirable products or side reactions, and can be produced on large-scales in a reproducible manner. In the subsequent sections we will discuss common chemical strategies utilized in the development of targeted NPs, mainly conjugation of a targeting ligand after NP formation and pre-conjugation of targeting ligands to polymeric precursors followed by self-assembly of NPs.

4.4.1 Chemical strategies for incorporating targeting ligands on NPs—The most traditional approach for the development of targeted NPs involves the conjugation of targeting ligands to the surface of NPs using facile coupling chemistries such as carbodiimide-mediated amide and maleimide-thiol couplings. Amide bond formation is a popular cross-linking reaction and carboxylic acids are frequently activated using a variety of carbodiimides such as 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). In this case, either the surface of the NP can bear carboxylic acids or the targeting ligand may have this functional group available for coupling. Although the formation of an amide bond using carbodiimide activation of carboxylic acids is a straightforward reaction, however, several side reactions can lead to undesired side reactions that produce *N*-acylureas, in addition to rapid hydrolysis of the reactive *O*-acylisourea intermediate. In order to avoid this hydrolysis an excess of the carbodiimide can be used, however considering that the surface carboxylic acid moieties are the solubility determining anchors of the NPs, then over-activation of these acids and formation of poorly soluble *O*-acylisourea intermediates can lead to a lack of solubility and colloidal instability and aggregation. These events can be minimized through the use of *N*-hydroxy-succinimide (NHS) carboxylic acid activated intermediates and can improve reaction efficiencies as the NHS intermediate is more resistant to hydrolysis. The formation of amide bonds between NP carboxylic acids and amino groups of targeting ligands is a popular bioconjugation method; however ligand conjugation numbers and

surface densities cannot be effectively controlled. Maleimide coupling with thiols is highly specific, less prone to hydrolysis, and can occur at neutral pH; this method has been very useful for coupling to cysteine residues of proteins or other thiol containing ligands, and avoids the formation of undesirable side reactions.[255, 256]

The search for highly specific chemical reactions that create unique linkages and avoid susceptibility to competing reactions has led to the development of "bioorthogonal" reactions. This class of reactions aremainly concerned with [3+2] cycloadditions between azides and alkynes, and is more commonly known as click chemistry.[257] In order to avoid the use of toxic copper catalysts in these reactions, various copper-free click chemistry reactions have also been developed and many studies are now underway that demonstrate the versatility of this type of bioconjugation technique.[258–261] A further bioorthogonal [4+2] cycloaddition reaction was recently described by Devaraj *et al.* and was applied to the labelling of cancer cells, peptides and small molecules.[262] This transition occurs between a 1,2,4,5-tetrazene (Tz) and *trans*-cyclooctene (TCO) and proceeds rapidly at room temperature and under physiological conditions without the need for a catalyst.[263] This conjugation reaction was successfully used in a novel labelling methodology termed "bioorthogonal nanoparticle detection" or "BOND" to conjugate antibodies to nanoparticles.[264] Antibody proteins were initially conjugated with TCO moieties and then reacted with Tz-functionalized NPs leading toNPs with multiple antibodies bound to their surface. This strategy can be used for efficient targeting and signal amplification and has been mainly applied to diagnostic applications for the highly sensitive detection of cancer cells.[263] From a synthetic perspective, the development of targeted NPs poses many challenges which include the following: (1) the exact ligand conjugation stoichiometry is not easy to control and therefore only average ratios of coupled ligands to NP surfaces can be estimated, (2) ligand conjugated NPs will not always have a low polydispersity and some NPs may possess higher numbers of ligands than others, (3) targeting ligands may not always attach to the surface of NPs through predictable covalent bonds and may interact with the surface of NPs through aggregation phenomenon, (4) the bioactivity of targeting ligands may be compromised following attachment to the NP surface, which could also affect the NP therapeutic performance, (5) the coupling chemistries involved must be highly specific, occur rapidly and ideally be amenable to biological environments, (6) the conjugation linkages employed between ligands and NPs must be durable in the highly complex *in vivo* environment and not become prone to competing reactions that may be brought about by changes in pH or hydrolysis unless these are intended mechanisms used for ligand and/or NP protective layer shedding and finally, (7) the bioconjugation techniques employed in ligand conjugation to NPs should be scalable, reproducible and economical.[265]

4.4.2 Targeted NPs through polymer self-assembly—As we have seen in the previous section, the conventional approach to the development of targeted NPs involves the conjugation of bioactive molecules to the surface of NPs *via* coupling chemistries. Even though excess amounts of reactants are often used to drive these reactions to completion, and given the heterogeneous nature of NP samples, it is difficult to control the stoichiometry of functional biomolecules on the surface of NPs, thus leading to poor reproducibility in manufacturing.

Recently, alternative approaches such as the self-assembly of targeted NPs using pre-functionalized components such as PEG polymers, and polymer-drug and polymer-targeting ligands have been developed to precisely engineer NP surfaces tending to uniformity (Fig. 6c).

The design of pre-functionalized triblock copolymers allows for the reproducible creation of optimal targeted NPs, whereby controlling the self-assembly and ratio of each constituent

can lead to targeted polymeric NPs with precisely tuned biophysicochemical properties.[196] Fig. 7 presents the development and characterization of PLGA-PEG-Apt triblock polymers, and the self-assembly of targeted NPs produced by the nanoprecipitation of a mixture of PLGA-PEG, PLGA-PEGApt and Dtxl drug. The concept of modular self-assembly of pre-functionalized material was demonstrated by Gu *et al.* who conjugated the A10 2′-fluorophyrimidine RNA Apt (Apt which binds to PSMA on prostate cancer cells), to PLGA-PEG block polymers in order to create triblock polymers that form targeted NPs by macromolecular self-assembly in a single step.[218] In this study, targeted NPs with varying ligand surface densities were developed which led to the identification of the exact narrow range of Apt density yielding the highest tumour accumulation *in vivo*. Interestingly, there was a non-linear inverse relationship between ligand density and tumour targeting *in vivo* and discordance between *in vitro* and *in vivo* optimal ligand densities. While *in vitro* data demonstrated enhancement of cellular binding and uptake as ligand density increased, the *in vivo* data demonstrated that tumour accumulation increased with the addition of ligands only to a certain point. As ligand density continued to increase on the surface of NPs, there was a paradoxical reduction in tumour accumulation and in parallel an increase in liver accumulation. Therefore, while the NPs were becoming more targeted in nature with increased Apt ligands on their surfaces, their surface was also becoming less stealth-like, resulting in rapid liver accumulation and poor EPR. These findings, for the first time, demonstrated the importance of narrowly optimizing NP biophysicochemical properties for *in vivo* success and clinical translation, and formed the foundation of the discovery and development of BIND-014 and SEL-068 targeted NPs (Fig. 1 and section 3.3).

Recently, Valencia *et al.* investigated the self-assembly and properties of two distinct targeted NPs (Fig. 8) decorated with two widely used small molecule targeting ligands with varying solubilities (folate and RGD).[266] While RGD is relatively hydrophilic, the folate molecule is relatively hydrophobic. Two different triblock copolymers of PLGA-PEG-RGD and PLGA-PEG-folate were synthesized using NHS/EDC amide coupling chemistry. NPs were developed using the nanoprecipitation method and this procedure demonstrated the ability to develop targeted polymeric NPs with different ligand densities by varying the ratios of the ligand bearing triblock polymers relative to PLGA-PEG copolymer lacking the targeting ligands. As predicted, further studies revealed the RGD targeting molecules to preferentially reside on the surface of the NPs, and the theoretically predicted RGD density on NP surfaces was shown to correlate to the experimentally derived RGD density. In contrast, the experimentally derived folate ligand density on NP surfaces was only 20% of the theoretically predicted folate density, presumably due to the fact that during the NP self-assembly process folate is in-part trapped in the hydrophobic NP core and cannot be presented on the NP surface. These results revealed the impact that ligand chemical properties can have on the targeting capabilities of self-assembled targeted PLGA NPs, and underscore the challenges associated with developing suitable targeted NPs and the need to optimize NP biophysicochemical properties on an individual basis.

Given the importance of the nature of the targeting ligands used in the development of targeted NPs, the various forms of targeting ligands, the isolation of high affinity ligands and ligand properties will be further discussed in the subsequent section.

5. Targeting ligands

Representative ligands for targeted NP development mainly include mAbs and their fragments, proteins or protein-like molecules, peptides, nucleic acid ligands (including Apts), small molecules and sugars.[122] In the following section we firstly look at specific examples using various ligands to summarize recent efforts in the development of ligand-based targeted NPs, and secondly we highlight strategies to isolate these targeting ligands

for use in a variety of medical applications. Additionally, various primary ligand properties that affect NP targeting will also be discussed.

5.1 Antibodies and their fragments

As discussed previously, mAbs have been most commonly used in the development of targeted NPs. For example, trastuzumab and rituximab which are mAbs currently in the clinic have been conjugated to PLA NPs resulting in conjugates that exhibit a 6-fold increase in the rate of particle uptake compared with similar particles lacking mAb targeting molecules.[267, 268] Nevertheless, mAb-targeted NPs still encounter many challenges and limitations since these molecules are large (~150 kDa), unstable in organic solvents (not amenable to incorporation on NP surfaces by self-assembly), are potentially immunogenic resulting in rapid NP clearance, and create engineering challenges for NP scale-up and manufacturing.[269, 270] Additionally, with an average hydrodynamic size of ~ 15 nm, when conjugated to NP surfaces they have the tendency to increase overall NP sizes by up to 30 nm.[141, 142] Therefore, antibody fragments including antigen-binding fragments (Fab) and scFv's have been developed to reduce these undesirable effects.[51]

Affibody molecules are small polypeptides derived from an antibody binding domain of *staphylococcal* protein A, that are used as scaffolds for the construction of combinatorial phage libraries.[271] From these libraries, affibody variants that target a specific cell marker can be selected using phage display technology. A 6 kDa affibody molecule has been reported with selective binding to the HER2 receptor with subnanomolar affinity.[140] Alexis *et al.* conjugated this affibody to the surface of polymeric NPs and showed increased uptake of these particles as compared to non-targeted NPs by breast cancer cells.[140]

Nanobodies are fully functional antigen-binding fragments evolved from the variable domain of heavy-chain antibodies. Similar to affibody molecules, nanobodies are also low molecular weight <15 kDa polypeptides that show similar antigen binding affinity which rivals that of the traditional mAbs.[272] Nanobodies are typically evolved from single-domain antibodies (antibodies carrying only a functional heavy chain without the light chain). In comparison with mAbs, these smaller molecules have been shown to have lower immunogenicity. Recently, a 15 kDa nanobody was isolated that demonstrates specific uptake *in vitro* and *in vivo* using xenograft-tumour-bearing mice expressing a carcinoembryoic antigen.[273]

5.2 Proteins

Many endogenous proteins that selectively bind to specific membrane-bound receptors on cells can be used for targeting *via* receptor-mediated endocytosis.[274] For example, the iron-transporting protein Tf, which binds specifically to the TfR, has been used to deliver NPs into different cell types. Choi *et al.* discovered that ligands targeting the TfR exert their influence by increasing uptake of targeted NPs by cancer cells and not by increasing particle accumulation in the tumour region.[275] Alternatively, growth factors such as epidermal growth factor (EGF)[276] and neural growth factor (NGF)[277] attached to NPs are other examples of protein-targeted NPs which have been shown to enter cells and elicit specific molecular responses. Proteins however, are commonly immunogenic and susceptible to early clearance by different mechanisms in the body, which may limit their effectiveness. Additionally, the receptors of these protein ligands are endogenous and commonly expressed on many types of non-targeted cells, which can lead to off-target adverse effects.[300]

5.3 Peptides

Peptides have gained interest as targeting ligands due to their small size, relatively low immunogenicity as compared to larger proteins, high stability, and ease of conjugation to polymers, lipids and NP surfaces.[301] Specifically, the development of highly specific peptide phage libraries, bacterial peptide display libraries, plasmid peptide libraries, and new screening technologies have made possible the synthesis of peptide ligands to a myriad of targets.[302, 303] Combinatorial libraries have led to the discovery of short peptides (10–15 amino acids) that are able to bind to targeted proteins, cells, or tissues specifically. The most studied peptide sequence is that of RGD which binds to αvβ3 integrin receptors which are highly upregulated on both tumour cells and angiogenic endothelial cells.[304] RGD is chemically synthesized, and is commercially available with different choices of linkers that allow for its conjugation to NP surfaces, biomaterials, or drugs. Despite the excitement of RGD-targeted therapies, some challenges still remain, including nonspecific adhesion, as αvβ3 integrin is also expressed on normal tissue and non-cancerous inflamed tissues.[304]

Development of phage display screening methods has successfully isolated peptide ligands with high specificity and affinity to various targets such as injured vasculature,[147] cellsurface hormone receptors such as luteinizing hormone-releasing hormone gene receptors (LHRHR),[305] and tumour vasculature antigens.[306] For instance, Chan *et al.* developed a NP system that targets vascular antigens exposed in cardiovascular-related diseases such as angioplasty-injured vasculature (Fig. 9, discussed in more detail in section 7).[147, 307] Similarly, Arap *et al.* have isolated peptides that bind specifically to tumour vasculature in patients and shown the potential of such *in vivo* phage library selections.[308] Other examples of peptides used to develop targeted NPs include Cys-Arg-Glu-Lys-Ala (CREKA),[221] Asn-Gly-Arg (NGR),[309] and Ile-Thr-Asp-Gly-Glu-Ala-Thr-Asp-Ser-Gly (LABL).[186] As discussed in section 3, the arrival of targeted NPs directly to tumour cells may be impeded by poor vascularisation and permeability within certain tumour regions. This problem is further confounded by the high interstitial pressure of tumours due to the leaky nature of their blood vessels and their dysfunctional lymphatic vessels which can diminish tumour tissue penetration and retention of NPs.[310] To further retain NPs within tumour tissue and to enhance cellular uptake, cell-penetrating peptides have been used to facilitate translocation of cargoes across the plasma membrane and to specific organelles within the cell.[311] Many of these sequences are derived from natural sequences, such as the protein-transduction domains of viruses.[274] For example, the Tat peptide derived from the HIV-1 virus has been popularly used to deliver a variety of NPs into cells. In particular, Tat-conjugated fluorescent quantum dots were used asmodel systems to investigate the trajectory of Tat-functionalized NPs within cells.[312]

While the Tat cell penetrating peptide does not display cellular specificity,[313] recently, other classes of peptides that interact with proteins upregulated on tumour vessels have been discovered. These peptides may ultimately assist the extravasation of NPs into tumours. Three representatives of this new class of peptides are: (1) iRGD, which binds αnβ3 integrins upregulated in tumours on the endothelial cell surfaces,[314] (2) LyP-1, which targets lymphatic vessels in some tumours,[315] and, (3) F3, which is an N-terminal fragment of human high-mobility group protein 2 that can bind to tumour vasculature.[316] All three peptides contain the cell penetration motif, R/KXXR/K or CendR sequence that is thought to interact with neurophilin-1 facilitating extravasation, tissue penetration, and cell entry of NPs.[314] Although high levels of these peptides have to be administered (*e.g.* reaching >100 μM levels in blood), it has been reported that this approach can potentially increase tumour uptake of a wide spectrum of small to large agents including NPs by several fold.[151] For example Sugahara *et al.* showed that systemic injection of iRGD peptide enhanced cancer drug delivery and activity when this peptide was administered as a combination therapy (without chemical conjugation) with either Dox, nab-Ptxl, Dox-liposomes or trastuzumab

mAbs, suggesting this combination therapy to be a valuable method to enhance anti-cancer drug efficacy.[281]

5.4 Nucleic acid ligands

Nucleic acid ligands (*i.e.* Aptamers (Apts)) are oligonucleotides that fold by intramolecular interactions into unique conformations with ligand-binding characteristics.[317] Apts can be DNA or RNA oligonucleotides, or modified oligonucleotides resistant to nuclease degradation. Apts can be selected from large pools of random oligonucleotide libraries by virtue of selective binding to antigens with high specificity and affinity. The *in vitro* Apt selection process is referred to as Systemic Evolution of Ligands by Exponential enrichment (SELEX) and was described independently by the groups of Larry Gold[318] and Jack Szostak[319] in 1990. Apts isolated from the SELEX process have approximate molecular weights of ~15 kDa, are chemically stable in a range of solvents, pH, and can be developed by chemical processes such as solid phase synthesis.[320–323]

NP-Apt conjugates targeting the PSMA were originally developed several years ago.[282] In these studies the Apt used as the targeting ligand was A10 Apt, first isolated by Lupold *et al.*[35] These targeted NPs were loaded with Dtxl and evaluated *in vivo* in a tumour model of LNCaP prostate cancer cells which overexpressed PSMA antigens. It was shown that the administration of these targeted NPs led to effective tumour size regression, following a single intra-tumour injection over a 109-day study.[124] In another study, Bagalkot *et al.* demonstrated a novel strategy for the targeted delivery of anthracyclic agents including Dox, directly to cancer cells through the formation of an Apt-Dox physical conjugate.[324] Recently Guo *et al.* presented studies that suggest PLA-PEG NPs targeted with the AS1411 Apt could cross the BBB and target nucleolin, a protein highly expressed in the plasma membrane of cancer cells, and specifically on glioma cells.[325] One key characteristic that endows Apts with high specificity against targets is their secondary structure that arises from their specific nucleotide sequence. However, this secondary structure may be affected by heat, exonuclease or endonuclease degradation, and other environmental factors which could limit the stability of Apts and their binding properties.[326]

5.5 Small molecules

The use of small molecules to create targeted NPs remains an attractive targeting strategy given their small size and ease of handling (less prone to degradation than biomolecular ligands). Some further advantages of small molecule targeting ligands include: (1) the availability of a range of facile coupling chemistries for their conjugation, (2) achievement of higher ligand densities on NP surfaces due to the small size of ligands, (3) availability of a wide range of targeting ligands with variable solubilities and functional groups, as facilitated by advances in diversity-oriented synthesis, (4) less immunogenic effects *in vivo* (compared to macromolecular ligands) and, (5) reproducible and scalable manufacturing.[327, 328]

One of the most extensively studied small molecule targeting moieties for drug delivery is folic acid (or folate). This highaffinity vitamin is a commonly used ligand for cancer targeting as folate receptors (FRs) are frequently over-expressed in a range of tumour cells. Folate specifically binds to FRs with a high affinity (Kd = ~10^{-9} M), enabling a variety of folate derivatives and conjugates to deliver molecular complexes to cancer cells without causing harm to normal cells.[329] For example, EC145 is conjugate of folate and a vinca alkaloid (desacetylvinblastine monohydrazide (DAVLBH)) currently in phase III development for cancer treatment.[330, 331] In addition to drug conjugates, folate has been used as a targeting moiety combined with a wide array of drug delivery vehicles (including liposomes, protein toxins, polymeric NPs, linear polymers, and dendrimers), to deliver drugs

selectively into cancer cells using FR-mediated endocytosis.[332] Given that FRs are expressed not only in tumour tissue but also in normal epithelia in the choroids plexus, placenta, lung, intestine, and kidney, FR-targeted NP delivery systems need to be further refined to increase tumour selectivity.[333] For example, as discussed previously (section 4.6) the optimal density of the targeting ligand should be investigated on a case-by-case basis given that this parameter affects the targeting efficacy of NPs to target cell populations. In these cases, greater ligand density does not necessarily translate to higher levels of tumour concentration—as NPs tend to lose their "stealth" surface characteristics and become increasingly prone to sequestration by cells of the MPS.[196] Therefore, optimal folate density on NP surfaces needs to be investigated and determined on a case-by-case basis.[334] Additionally, folate is relatively hydrophobic in nature and strategies to maximize its binding properties on the NP surface need to be carefully considered and investigated.[266] Additionally, an increased surface density of folate ligands on NP surfaces has been shown to give rise to dimers, trimers or tubular quartet self-assembled folate structures which can hamper the binding efficiency of folate to its receptors.[335]

In one study, Pomper *et al.* were able to identify a small hydrophilic molecule that could target the PSMA receptor in prostate cancer cells.[336] They showed that this molecule induced NP internalization by cells through endocytosis and preferential accumulation of NPs in tumours overexpressing PSMA receptors. Given that PSMA is not only overexpressed on the surface of prostate cancers but also on the neovasculature of all solid tumours, this targeting strategy is very attractive for therapy and diagnosis applications.[337, 338]

Carbohydrates, or sugar moieties, have also gained attention as targeting ligands due to their ease of production, low molecular weight and high abundance in nature. Some of these carbohydrate ligands target the membrane carbohydrate-binding proteins (membrane lectins) differentially expressed on the cellular and intracellular membranes of a number of cells. Their multiplicity, high affinity, and effective endocytosis after receptor binding as well as the biocompatibility of carbohydrate ligands make them potentially suitable ligands for carriers in cell-selective delivery of drugs and nucleic acids.[339] Among the different targeting ligands mannose,[340] glucose,[341] galactose,[342] and their derivatives have been successfully tested.

5.6 Ligand selection

5.6.1 Selection of high affinity ligands against complex targets—As a result of the need for highly efficient targeting ligands for use in a variety of biomedical applications as well as conjugation to NPs, a number of novel ligands with high affinities, internalization and transcytosis capabilities have been isolated recently, examples of which are presented in Table 3. The majority of the ligands currently available for targeting applications have been developed against well characterized purified proteins. However, selections against purified proteins are hampered by the limited number of purified receptors available, especially when the protein targets are insoluble or the targets are functionally part of multiprotein complexes.[343] To overcome these limitations, selection protocols based on living cells have been developed as alternative methods.[344, 345] In contrast to the selection processes against purified proteins, cell-based selections can be performed without prior knowledge of targets or multi-protein complexes expressed on the cell surface. Moreover, intact living cells with many native receptor proteins can be used as targets during the selection procedure. This allows for a variety of ligands targeting several proteins on the same cell to be isolated from such screenings.[346] Since this strategy relies on the differences between the target cell population and the control cell population used for counter-selection (*e.g.* defined phenotype, protein expression levels, different protein conformations), multiple ligands

recognizing only target cells and not the control cells can be identified. For example, de Kruif *et al.* extended phage display technology to living cells, whereby they isolated human scFv antibodies specific against subsets of blood leukocytes, and obtained two phage antibodies from such screening with specific binding to B-lineage cells.[345] Similarly, Shangguan *et al.* successfully isolated a panel of Apts that can distinguish leukemia T-cells from B-Cells using cell-based SELEX.[347]

Given that the binding of ligands is dependent on their target's conformation, which in turn is affected by the target's environment, selection strategies that can generate targeting ligands capable of specifically localizing to tissues or organs *in vivo* have been developed. In 1996, Pasqualini *et al.* introduced the technique of *in vivo* phage display and screened a group of peptides capable of specifically localizing to brain and kidney blood vessels.[348] Similarly, this *in vivo* screening method was conducted to isolate prostate tissue-homing peptides.[349] More recently *in vivo* phase display and screening was performed on 8 live patients with stage IV cancer resulting in the identification of several tumour-homing peptides and scFVs.[303] In 2010, Mi *et al.* designed an "*in vivo* selection" approach to screen a library of nucleaseresistant RNA oligonucleotides in tumour-bearing mice in order to identify candidates with the ability to localize to hepatic colon cancer metastases. One of the selected molecules was an RNA Apt that binds to p68, an RNA helicase that has been shown to be upregulated in colorectal cancer.[350] Further efforts are now underway to take these selection processes to the next level—that of identifying ligands capable of specific cellular internalization and transcytosis (transport across the cell interior).

5.6.2 Selection of cell internalizing and transcytosis ligands—Considering that intracellular delivery of drug loaded NPs could provide enhanced therapeutic effects over NPs that do not enter cells, selection techniques based on receptor-mediated endocytosis have been developed to identify internalizing ligands that are useful for NP delivery. For example Burg *et al.* identified eight distinct prostate cancer (PC3) cell internalizing peptides from a diverse peptide-display library.[296] Similarly, a subset of internalizing scFv antibodies has been identified by phage antibody selections against PC3 cells.[297] These antibodies have been further conjugated to NPs to direct their specific delivery into PC-3 cells.[297]

Recently, Xiao *et al.* developed a "cell-uptake selection" strategy to isolate a group of cancer-cell specific internalizing RNA Apts (Fig. 10).[351, 352] In this strategy, selection was carried out against PC3 or LNCaP prostate epithelial cell lines to identify Apts that differentially bound to either PC3 or LNCaP and were subsequently internalized. In order to increase the specificity of the selection process, several cell lines representing normal prostate and non-prostate cells were used in the counter-selection process to deplete the Apt library from ligands that also interacted to these other cell types. Different from previously reported Cell-SELEX processes, this selection was performed at physiological temperature (37 °C), where cells and their membrane receptors are biologically active and continue their endocytosis functions. In addition, the isolated RNA Apts were introduced with 2′ O-methyl (OMe) modifications during the selection process, which facilitates the resistance of nuclease degradation inside intra-cellular environments. More importantly, only internalizing Apts were selectively collected, since the non-internalized membranebound Apts were removed during the selection process. After 12 rounds of selections, a group of PC3 or LNCaP specific internalizing Apts were identified and further conjugated with polymeric NPs. Results demonstrated that the internalizing Apt-targeted polymeric NPs were specifically and efficiently taken up by targeted cells, and could drastically improve the cellular cytotoxicity of Dtxl compared to non-targeted NPs.

Towards the delivery of NPs capable of crossing physical barriers, such as the BBB, transcytosis peptide ligands have been isolated by utilizing phage display techniques. Wan *et*

al. screened a C7C phage display library of peptides that were intranasally administered to rats, and subsequently recovered phage from the brain tissue.[298] This screening revealed a peptide sequence (ACTTPHAWLCG) that can bypass the BBB through the nasal-to-brain passage.[298] Additionally, Rooy *et al.* selected two 15-amino acid-peptides (GLA and GYR) that can bind to the murine brain in an *in situ* brain perfusion model.[299] Recently Li *et al.* isolated TGNYKALHPHNG peptide (denoted as Pep TGN) through *in vivo* phage display screening, and when conjugated on the surface of PLGA NPs, Pep TGN was shown to facilitate targeted delivery of these NPs across the BBB, leading to significant higher cellular uptake and *in vivo* brain accumulation. [194] These studies demonstrate both the feasibility and efficiency of *in situ* ligand selection techniques and can be applied to a range of applications where there is a need for high affinity binding ligands that can direct NPs to disease cells.

5.6.3 Ligand properties affecting NP targeting—Efficient argeting of NPs to the cell surface lipid bilayer may be dependent on two important ligand properties, which include; ligand affinity, and ligand density. Upon binding of targeted NPs to the cellular lipid bilayer, complementary membrane receptors fluidly diffuse together to initiate NP membrane wrapping.[353] If the binding affinity of the ligand to its receptor is strong, sufficient thermodynamic energy is generated to overcome the elastic recoil of the cellular membrane back to its original equilibrium state resulting in membrane wrapping around NPs.[353] For large NPs, high binding affinities are particularly required to prolong the residence time of NPs onto cellular surfaces. In this situation, binding affinities are majorly influenced by equilibria between enthalpic advantages due to ligand-receptor binding and entropic losses for tether chain stretching or compressibility around the cell surface environment.[354] Controlling ligand-to-NP ratios has been traditionally difficult, leading to heterogenous distributions of ligands on the surface of NPs. In order to address this issue the effective self-assembly of pre-functionalized and pre-targeted tri-block polymers was accomplished by Gu *et al.*[196] This study demonstrated the facile tuneability of the density of ligands on NP surfaces, which was achieved by the blending of variable ratios of the targeted triblock polymers with other polymer blocks. In this way, the overall ligand density of NPs can be controlled leading to more homogenously targeted NPs. Tassa *et al.* recently quantitatively studied the affinity and binding kinetics of NP bearing small molecules using surface plasmon resonance (SPR).[355] In this study the interactions between a single protein target and a range of targeting ligands with variable intrinsic affinities was measured. It was concluded that even small ligands with weak affinities could still significantly enhance target-specific avidity *via* multivalent interactions. Knowledge of NP affinity and association and dissociation rates may ultimately predict how deeply NPs can penetrate into tissues once target cell populations become saturated.[355] Therefore, NP size, target ligand density and tissue permeability as governed by capillary permeability are all factors that affect the efficacy of NP targeting *in vivo*.

In another study, the effect of ligand density on the internalization of PLGA NPs was recently investigated using the cyclic peptide ligand, cLABL which binds to the intercellular cell adhesion molecule-1 (ICAM-1).[356] In this case an optimum of between 2 to 4 pmol cm^{-2} of the peptide ligand was found to be effective for targeting to A459 cells. Further discussions on the optimization of ligand densities can be found in a review by Pirollo *et al.*[145]

Throughout this review so far we have seen that the nature of the targeting ligand (*i.e.* whether small molecule or macromolecular ligands *etc.*), the size of the NP system and the degree of ligand density on the NP surface, are all factors that affect the binding efficiency of targeted NPs to target cell receptors or other antigens, and can alter the path of NPs *in vivo*. Given the wide nature of ligands and targeted NPs developed, at this point, ideal

generalizations of relationships between ligand density, NP size and optimal ligand binding affinities cannot be made and must be investigated for each unique system.

6. Optimal biophysicochemical characteristics of NPs

NP physicochemical properties, such as size, geometry/shape, surface charge, surface chemistry, hydrophobicity, roughness, rigidity, and degree of composition (Fig. 11), can result in differential uptake and/or targeting to certain organs, tissues or cells and may be optimized through the effective design of NPs.[357] In this section, we will focus on discussions of these primary properties of NPs, the optimization of which is essential for the development of efficient therapeutic NPs.

6.1 Influence of NP size

NP size is a key parameter affecting the cellular uptake rate of NPs as it influences their internalization mechanism, and it is also a key property for *in vivo* circulation half-life as discussed further below.

There are two major endocytic mechanisms by which cells take up particles and macromolecules, and these are referred to as phagocytosis and pinocytosis (or fluid-phase uptake).[358] Large particles (>1 μm) are generally internalized by phagocytosis mechanisms, which are present only on professional phagocytic cells, such as macrophages, neutrophils, or dendritic cells.[359] Therefore pinocytosis is more relevant to NP cellular uptake and can occur either *via* adsorptive pinocytosis (non-specific adsorption of NPs or macromolecules to the cell membrane followed by internalization) or *via* receptor-mediated endocytosis (RME, which describes the interaction of NPs andmacromolecules with receptors, followed by their internalization).[360] Pinocytic mechanisms of uptake can be further divided into caveolae-mediated endocytosis or clathrin-mediated endocytosis, as well as clathrin-independent or caveolin-independent endocytosis (smaller NPs can be internalized through a number of these pathways).[359] Detailed discussions on these pathways and their implications for NP cellular uptake can be found in other excellent recent reviews.[359–363]

Cellular internalization of nanoparticles is majorly dependent on the size of the NPs, and in general, particles in the 40–50 nm range exhibit maximal uptake *in vitro*.[349, 362] Jiang *et al.* studied the interactions of targeted antibody conjugated silver and gold NPs and found that 40–50 nm particles exhibited the highest amount of cellular internalization and concluded that this optimal size range for NP uptake is likely due to an intricate balance between multivalent cross-linking of the membrane receptors and membrane wrapping processes taking part in RME.[364] In this study Herceptin (Her) gold NPs (2–100 nm) were synthesized and the size-dependent binding and uptake of these NPs was investigated with ErbB2 receptor expressing cells.[364] It was also shown that the number of Her antibody binding sites on the NPs was dependent on the NP surface area and increased with particle radius. Antibody density on the surface of the NPs also increased linearly with NP radius—demonstrating that these multivalent antibodyconjugated NPs can allow for a high degree of ErbB2 cross-linking which can be tuned by NP size.

In general, 10–100 nm is a generally accepted size range for the development of NPs for *in vivo* applications which relates to their *in vivo* clearance and biodistribution patterns.[360] These upper and lower bounds are largely determined by interactions with the immune system and kidney filtration cut-offs, respectively. Larger NPs possess low radii of curvature which can lead to increased interactions of opsonins onto their surface and faster clearance rates *in vivo*.[365] The predominant proteins involved in the opsonization process are plasma complement and immunoglobulin proteins which can lead to development of hypersensitivity towards NPs.[366] Large NPs are prone to filtration through the sinusoids in

the spleen and clearance by the MPS cells, which include the Kupffer cells of the liver.[367] Additionally, NPs smaller than approximately 5.5 nm have been shown to be rapidly cleared by glomerular filtration in the kidneys.[368]

For the purposes of tumour accumulation, the upper limit for extravasation into solid tumours has been suggested at ~400 nm and it is generally observed that NPs <200 nm in size can accumulate effectively within tumour tissue, with the 70–200 nm range considered optimal for tumour passive targeting.[369] However, recent studies have revealed that there is an optimum size for maximum uptake by various tumour cell types, and even a 10 nm deviation from this optimum results in a significant decrease in NP uptake.[364] Concurrently, some researchers have found that NPs of about ~10–20 nm are ideal for maximum tumour penetration.[370]

Recently, a study by Schadlich *et al.* investigated the size dependent accumulation of fluorescently labelled PLA-PEG polymeric NPs using two different tumour xenograft models, HT20 colon and A2780 ovarian carcinoma, which result in different tumour structures, growth rates, and microenvironments. [371] Using an *in vivo* fluorescence imaging technique, the biodistribution and accumulation of NIR-loaded PLA-PEG NPs was tracked. NPs 111 and 141 nm's in diameter were shown to accumulate efficiently in tumours and the larger NP (166 nm) was observed to undergo rapid clearance in the liver. The pattern of accumulation was shown to be different in both tumours, however, fluorescence was mostly observed from the tumour core region in the case of HT29 tumours, which was not observed in the A2780 tumours. Using different NP size batches the authors concluded that NP accumulation to the necrotic HT29 tumour core is size independent, but size dependent in the more vascularised A2780 tumours. Additionally, larger NPs led to lower tumour accumulation. Interestingly, this study concluded that size variations of between 20–30 nm (z-averages) led to highly distinct *in vivo* outcomes for NP distribution. Although the NPs used in this study utilized NPs with low polydispersity indexes (PDI: 0.013–0.16), these results help to shed light on the importance of tumour vascularity and narrow NP size distributions, since polymeric NPs can have high PDIs due to inherent variations in both the molecular weight of the polymers and the mixing time of precursors during preparation.[51]

With regards to polymeric NPs, our previous work has demonstrated that the molecular weights (MW) of PLGA together with the concentration of polymer in organic solution are key parameters that allow for independent tuning of NP size.[372] We have also successfully demonstrated that NPs made from a PLGA-PEG polymer with PLGA MW of 15 KDa at low concentrations in microfluidic devices can be formed in the range 20–25 nm.[236] Similarly, NPs made from PLGA-PEG with PLGAMWof 95 KDa at high concentrations were achieved in sizes of 200–250 nm.[372] These promising methods demonstrate the feasibility of obtaining small sizes in addition to highly narrow PDIs and demonstrate the use of microfluidics to effectively devise libraries of homogenous NPs with a range of sizes.

Given the range of nanomaterials and cell types used to study the effects of NP size on cellular uptake rates and mechanisms, and the often contradicting results and claims that has been demonstrated in the literature so far, then it is important for an optimal NP size to be determined experimentally for a given NP and specific cell type. Furthermore, so far not many studies have been conducted that investigate the relationship between incorporation/ density of targeting ligands and NP size on cellular uptake efficacy and indeed these studies merit further investigation.

6.2 Influence of NP shape

Recent studies have shown that particle shape may be an important factor in the rate of NP cellular internalization.[362] This is mainly due to the fact that NP shapes that can

accommodate cellular membrane wrapping processes become most effective at cellular uptake. Studies have shown that amongst NPs of either rod or sphere design, the spherical shaped NPs were taken up by cells more readily.[361] In another example, Desimone *et al.* have produced a variety of NP shapes using their top-down fabrication method termed particle replication in nonwetting templates (PRINT) (Fig. 12).[360] They found that the internalization of rod-like NPs with high aspect ratios (depth: 150 nm, height: 450 nm and volume: 0.00795 μm^3) occurs faster in HeLa cells than that of cylindrical NPs regardless of NP volume.

NP shape is also an important factor for the biodistribution and circulation of NPs *in vivo*. Geng and Decuzzi *et al.* have reported that non-spherical particles with longitudinal lengths reaching cellular diameters and discoidal shapes can exhibit longer circulation times than spherical particles.[373, 374] Given the typical processes used to fabricate therapeutic NPs (bottomup fabrication, self-assembly), the majority of these particles are spherical. As such studies determining optimal NP shapes are still at an early stage and further investigations are required to determine the effects of NP shape on cellular uptake.

6.3 Influence of NP surface charge

NP surface charge is a major factor contributing to the nonspecific binding of NPs to cells and proteins in blood circulation. Positively charged NPs are rapidly cleared from circulation by cells of the MPS.[360]

NPs with cationic surfaces may promote cellular binding, resulting in either uptake through endocytosis or direct penetration of the cellular surface membrane. This is because cationic surfaces will interact with the negatively charged phospholipid head groups, proteins and glycans on the surface of cells.[359] Similarly, negatively charged NPs can also show selective cellular uptake compared to NPs with neutral surfaces.[361] Indeed, NP surface charge is a predominant factor for endocytotic uptake of NPs into cells. Recent studies have shown the uptake of positively charged NPs to be an energy dependent process involving the proteins dynamin and F-actin, whereas negatively charged NPs were internalized in a dynamin-independent manner.[375] On the other hand, highly cationic NP surfaces could also by-pass endocytic modes of entry into cells as they can enter cells by creating holes in the cellular bilayer.[359]

Charged NPs however, will inevitably have short half-lives *in vivo* and high non-specific cellular uptakes due to interaction with blood proteins, resulting in complement activation. Indeed a recent systematic study of the immunocompatibility properties of lipid-polymeric NPs was conducted by measuring the effects of carboxy, amino and methoxy terminated PEGylated NPs on the degree of complement system activation, human plasma protein binding and coagulation system activation.[376] Amongst the surface functional groups studied, amino terminated surfaces induced the highest levels of complement activation. NPs with the more neutral methoxy surface groups were most immunocompatible. Another study showed that after systemic administration, particles with surface charges <15 mV showed minimal macrophage uptake and led to longer circulation times and hence tumour retention.[363]

From our previous experiments we have concluded that the surface charge of NPs can be easily tuned by modifying the functional end group of the PEG polymer.[377] Specifically, NPs composed of PEG-NH_2 exhibited a zeta potential of 10 to 15 mV while NPs composed of PEG-COOH exhibited a zeta potential of −10 to −15 mV, and those composed of PEG-OCH_3 remained neutral. More interestingly, we have shown that by blending PEG polymers with different end groups at various ratios the NP surface charge can be modulated to a desired value.[376]

6.4 Influence of NP hydrophobicity, roughness, and rigidity

Hydrophobicity plays an important role in NP targeting to cells; NPs that are more hydrophobic than the cellular surface membrane are more easily taken up by cells.[359] Likewise, protein adsorption on the surface of NPs is highly dependent on the hydrophilicity of NP surfaces. Hydrophobic surfaces tend to lead to higher levels of protein adsorption, and IgG proteins which are also opsonins, have high affinities for hydrophobic surfaces.[378]

In addition, surface effects such as smooth *versus* rough surfaces also influence the degree of NP surface binding to cells.[379] Nanoscale "roughness" manifests as local protrusions or depressions on the NP surface forming a harsh surface that has been shown to lead to a minimization of repulsive forces between cellular and NP surfaces.[353] Nanoscale surface roughness influences the surface topography of NPs and was recently exploited to enhance the growth and osteogenic differentiation of human-bone-marrow-derived mesenchymal progenitor cells.[380]

Particle rigidity can highly influence *in vivo* biodistribution profiles. Red blood cell like hydroxyethyl acrylate hydrogels were synthetically engineered with both rigid and deformable structures and their biodistribution evaluated *in vivo*.[381] The more deformable synthetic blood cells were shown to eliminate up to 30 times more slowly than their rigid counterparts, with more rigid microparticles accumulating in the lungs 2 h post-injection. Given the highly flexible and deformable discoid shape of naturally occurring red blood cells, it is hardly surprising that the elastic modulus properties of NPs can also play an important role in the pharmacokinetics and biodistribution of particles, with more flexible NPs traversing vessels and pores more easily *in vivo*. Studies exploring the relationship between targeted NPs with variable degrees of structural rigidity and flexibility should be of high interest.

6.5 Influence of NP PEGylation

Due to their large surface-area-to-volume ratios, NPs can attract a 'corona' type binding of blood proteins to their extremely curved surfaces.[353] These numbers of proteins are by no means few and far between, but in the hundreds.[382] Although plasma protein binding by abundant proteins such as albumin and 1-acid glycoprotein may lead to enhanced bioavailability for traditional small drug molecules (through the reduction of first pass hepatic extraction), however in the case of NPs, this can lead to their enhanced blood clearance by cells of the immune system.[26] The binding of plasma proteins onto the surface of NPs, also known as opsonization, occurs instantaneously once the NPs make contact with the blood-stream. To address this issue, one classical design parameter for effective *in vivo* circulation and immune system shielding is the surface addition of PEG, termed PEGylation.[365] PEG is a highly hydrophilic polymer that ensures prolonged *in vivo* halflives. Indeed, uncoated NPs have been observed to be rapidly cleared by the MPS.[378] The density and thickness of this PEG masking layer has also been found to affect opsonization and biodistribution of injected NPs, and should be studied with more high-throughput and combinatorial approaches that can accelerate the discovery and development of NPs in a reproducible manner, together with a comprehensive study that combinatorially investigates the interrelation of NP PEG lengths and densities leading to reduced clearance.[365, 384] Currently, it is common practice to decorate the surfaces of NPs with PEG polymers, and these types of NPs can benefit from prolonged circulation times.[385] As such, surface-grafted PEG NPs have reduced uptake by liver cells as these NPs are not effectively bound by plasma proteins.[386] In addition, the density and configuration of PEG on the surface of NPs is also an important parameter for *in vivo* biodistribution. PEG configurations on the surfaces of NPs can exist as either extended brush-like structures or coiled mushroom or mushroom/brush intermediates. From the two configurations,

predominant brush-like PEG surfaces have been shown to sterically suppress the approach and binding of opsonins such as the C3 protein.[378]

Studies have shown that NPs covered with PEGMWof 10 kDa circulate longer in the blood but accumulate less in the tumour, while NPs with PEGMWof 5 kDa remain for shorter duration in blood circulation but accumulate more in the tumour.[387] In addition, there are articles that intend to elucidate an "optimal PEG coverage" that falls in between a larger number of PEG chains on the NP surface with low mobility (brush configuration), and a lower number of PEG chains on the NP surface with high mobility (mushroom configuration).[388]

In summary, the diversity involved in NP biophysicochemical properties significantly affects their cell uptake, cell cytotoxicity, PK and BD *in vivo*. Additionally, there is a strong interplay between each of these properties and an optimal combination needs to be experimentally determined for everyNP type. Given the many parameters that must be optimally engineered and the variability and polydispersity in properties due tomultiple synthesis steps, it is not surprising that optimal biophysicochemical properties of NPs have been difficult to ascertain, as evidenced by the fact that many novel nanotechnologies have failed to make an impact on human health to date. Therefore, it is clear that there is a need for optimization of NP biophysicochemical properties in order to achieve optimal efficacy. Interest in the exact identification and characterization of NP surfaces is on the rise and computational techniques including electronic structure methods, all atom Monte Carlo, molecular dynamics methods and coarse-grained methods are now in use in order to better predict and understand the dynamic interactions of NP surfaces with biological environments and systems.[389]

7. Novel trends in the development of targeted polymeric nanoparticles

7.1 Multi-targeting

Multi targeting systems (MTS) generally describe NP surfaces decorated with two or more targeting ligands that recognize different receptors on the same or different cells. This type of targeting has gained a substantial amount of interest as a way to increase NP uptake by cancer cells using a "two-punch" (or "three-punch") approach. An example of this approach was presented by Kluza *et al.*[390] where they conjugated RGD (targeting integrin receptors) and anginex (targeting galectin-1) to paramagnetic and fluorescent liposomes and investigated NP cell uptake. Interestingly it was found that RGD-anginex dual targeting synergistically enhanced the uptake of these liposomes by HUVEC cells. Another example is demonstrated by Li *et al.* who used two commonly utilized small molecules, folic acid and glucose, to target KB cells with overexpressed FRs (Fig. 13b).[341] In this work, the results suggested that enhanced cell recognition and internalization was due to increased multivalent interactions of the gold NPs with the cell surface, compared to single-ligand targeted NPs. Patil *et al.*[391] also prepared dual-targeted PLA-PEG NPs using folic acid and biotin as targeting agents and investigated the tumour accumulation and efficacy of this construct *versus* folate-targeted and biotin-targeted mono-targeted NPs. The targeting strategy resulted in improved efficacy and higher tumour accumulation when compared to controls. More recently Ashley *et al.*[383] developed porous silica NPs coated with a lipid bi-layer decorated with both a targeting peptide (SP94 which targets an unknown receptor in human hepatocellular carcinoma) and a fusogenic peptide (H5WYG, which enables endosome escape after being protonated) (Fig. 13c). This strategy resulted in a 10 000-fold greater affinity for hepatocellular carcinoma than for hepatocytes and 106-fold improvement over comparable liposomes. A further example of a novel targeting system was demonstrated by Sugahara *et al.* using iRGD.[281, 314] This cell penetrating peptide has the added property of targeting two different receptors on the same cell (integrin and

neuropilin-1 receptors) (Fig. 13d). In this case, targeting occurs sequentially, where integrins are targeted first with the iRGD moiety, followed by proteolytic cleavage of the peptide exposing a CendR motif that subsequently targets neuropilin-1, a key receptor for penetration of biological barriers.[281, 314] Additionally, when iRGD was conjugated to the surface of Abraxane (Albumin-paclitaxel NPs), the efficacy of the therapy in mice was improved by several folds.[281]

While dual targeting presents an attractive strategy to potentiate NP uptake by cancer cells, it brings to the NP design another level of complexity. For a targeted NP to be highly effective, all the physicochemical properties such as size, charge, surface hydrophobicity, and targeting ligand density need to be optimized. Considering the interplay of these NP properties and the difficulty to alter one without affecting the other, introducing multiple types of targeting ligands to the NP design could potentially further complicate the optimization of NPs. Furthermore, the greater the number of components a NP has, the more challenging its large-scale manufacturing, regulatory approval, and translation to the clinic. Although studies have shown that the presence of two different targeting ligands on a single NP led to potent cytotoxicity, whereas no toxicity was observed when only one type of ligand was used,[177] nevertheless, the ultimate benefit of dual or multi targeted NP systems should continue to be investigated at least at the pre-clinical stage.

7.2 Screening NP libraries

Small-molecule drug discovery is generally conducted by screening libraries of molecules to find drugs with optimal pharmacokinetics, biodistribution, tolerance, disposition, and elimination profiles. In recent years researchers have also taken this approach for the development of targeted NPs. However, targeted NP screening increases complexity by several orders of magnitude since there are several 'inputs' that could be screened for, such as chemical diversity from the precursors, or biophysicochemical diversity from the NPs. Careful design of combinatorial methods for measuring and understanding these diversities is one solution. For instance, Siegwart *et al.* recently developed a semi-automated screening of over 1000 polymeric and lipid-like materials with wide chemical diversities that could efficiently deliver siRNA.[392] Each material was formulated into a NP and gene silencing was evaluated *in vitro*, followed by *in vivo* evaluation of a set of 'hit' NPs.[392]

The utility of NP screening libraries was demonstrated by Hrkach *et al.* who developed a library of ~100 distinct targeted polymeric NP formulations varying with respect to particle size, targeting ligand density, surface hydrophilicity, drug loading, and drug release properties, leading to a range of NPs with narrowly controlled biophysicochemical diversities.[394] PK studies in mice, rat and monkey; and tissue distribution studies in rats showed that the lead targeted NP, BIND-014, had properties atypical of previously described polymeric NPs, including a blood circulation half-life of approximately 20 h and minimal liver accumulation.[394] BIND-014 demonstrated superior anti-tumour efficacy in animals, as well as differentiated pharmacology, tolerability, and promising anti-tumour responses in human clinical trial.[394]

Weissleder *et al.* reported the screening of 146 different small-molecule ligands attached onto a model NP.[393] Their model NP was a 'magneto-fluorescent' dextran coated iron oxide NP that could readily report uptake by different cell types, as well as tumour accumulation. Their choice of small molecule targeting was focused on compounds (MW < 500 Da) containing various chemical functional groups such as primary amines, alcohols, carboxylic acids, sulfhydryls and anhydrides, which can effect overall NP features such as water solubility, conjugation ability, biocompatibility and chemical diversity (Fig. 14).[393]

Wang *et al.* also screened various NPs to identify those with the best gene transfection efficiencies *in vitro*.[243] This system was composed of six different building blocks including two different targeting ligands linked to PEG, cyclodextran, and dendrimers, with the mixing ratio of the compounds varied combinatorially. One important feature of this work was the development and implementation of a microfluidic system that facilitated the rapid mixing and preparation of different building block combinations in a high-throughput manner.[243] These examples demonstrate a rising trend towards the combinatorial development of NPs to find NPs that exhibit the optimal interplay of properties impacting their efficacy and fate *in vivo*. The screening of NP libraries is one approach to comprehensively investigate the interrelation of NP biophysicochemical properties with their final therapeutic outcome.

7.3 Combination therapies

Combination therapy by co-delivering multiple drugs encapsulated in the same targeted polymeric NP was proposed to address the challenges of single-agent chemotherapy.[46, 395–397] Some of the advantages of this type of approach include: (1) synergistic therapeutic effects, (2) control of drug-combination pharmacokinetics (especially for drugs with different chemical properties) and, (3) control of combination-drug exposure in a temporal and spatial manner. Combination chemotherapy can be carried out in non-targeted NPs or targeted NPs.[46, 398] Recently, Kolishetti *et al.* developed a targeted therapeutic NP system for co-delivery of cisplatin and Dtxl (two drugs with different chemical properties) to prostate cancer cells.[46] The hydrophilic Pt(IV) cisplatin prodrug was first conjugated to a poly(lactide) polymer derivative with pendant hydroxyl groups (denoted PLA-OH) to yield a PLA-Pt(IV) copolymer, and subsequently blended with PLGA-PEG and Dtxl by a nanoprecipitation process (Fig. 15).[46] The dual-drug encapsulated NPs were then conjugated with the A10 Apt to develop a targeted co-delivery NP platform.[46] *In vitro* studies demonstrated that the Apt-targeted, dual-drug encapsulated NPs were ~5.5–10 times more cytotoxic than respective single drug encapsulating NPs (PLA-Pt-NP-Apt and Dtxl-NP-Apt).[46]

In another study by Milane *et al.* the therapeutic efficacy and safety of the drug combination paclitaxel/lodinamine loaded in an EGFR NP was evaluated.[396] The rationale for the targeting agent together with the drug combination was to overcomeMDRwith cells over-expressing the EGFR receptor.[396] The drug combination was evaluated using a mouse orthotopic model of MDR human breast cancer, and it was observed that the NP performed significantly better than the free drug combination.[396] Research on the development of combinatorial therapies is on the rise, however, this area will benefit from further investigations involving: (1) the discovery of further molecular targets in cancer cells and better understanding of drug activity in these cells, (2) chemistries that allow for the dual or multi-conjugation of drugs to polymer backbones, (3) improvements in loading methods of two or more drugs with variable properties within single NPs and, (4) reproducible and efficacious toxicity data following the use of combination drug therapies.

7.4 Polymer-lipid hybrid NPs

Polymer-lipid hybrid NPs aim to combine the advantages of both polymers and lipidic vesicles and have recently gained interest for drug delivery applications. The main rationale concerning the design of these systems is to achieve stable and controllable NPs for prolonged drug release properties *in vivo*. This involves the inclusion of an amphiphilic lipid in the formulation, which preferentially associates with the hydrophobic corona surface of the polymeric core, for example a PLGA based core (see Fig. 9). Recently Chan *et al.* developed PLGA-lipid NPs that combined three different types of biomaterials: (1) a PLGA hydrophobic core for drug entrapment, (2) soybean phosphatidylcholine (lecithin) to create a

monolayer around the hydrophobic core, and, (3) DSPE-PEG-COOH which intersperses in the lecithin monolayer in order to form a stabilizing PEG shell (Fig. 9).[399] These polymeric NPs were loaded with Ptxl and conjugated to peptides where they showed effective vascular wall targeting and preferential localization to arterial wall injury. Additionally a post-angioplasty reduction in arterial stenosis was observed in animals treated with these Ptxl-load vascular wall targeted NPs.[147, 307] Another example of combining polymers and lipids in a single delivery system is the "nanocell" developed by Sengupta *et al.*[188] In this work, the authors were able to achieve delivery of two anticancer drugs in a spatiotemporally controlled manner by incorporating a polymeric nanoparticle inside of a liposomal structure. The liposomal portion released its antiangiogenic payload more rapidly, thereby attacking the tumour-associated vasculature, followed by a more sustained release profile of a cytotoxic agent from the inner polymeric matrix, directly attacking the tumour cells.

Recently Shi *et al.* reported the development of a simple and robust hybrid lipid-polymer NP for siRNA delivery.[400] This system differs from classical lipid-hybrid polymer systems in that these NPs were engineered to contain a differentially charged hollow core/shell lipid-polymer-lipid hybrid nanostructure (Fig. 16). These NPs were formulated using a modified double-emulsion solvent evaporation method. The inner positively charged hollow core which is composed of cationic lipids was designed to encapsulate siRNA more efficiently than a PLGA core alone. These hybrid lipid-polymeric NPs were shown to effectively load and efficiently deliver siRNA both *in vitro* and *in vivo*.

Hybrid NPs, especially those made with clinically validated materials hold great promise as the next generation of therapeutic NPs. The fact that hybrid NPs combine some of the unique physical and chemical characteristics of two or more classes of materials, such as polymers and liposomes to create a versatile and robust new class of nanoparticles, make them very attractive to potentiate the performance of NPs. However, it would be key to obtain a deeper scientific understanding on hybrid NPs, both physical (for instance understanding their self-assembly mechanism) and biological (for instance, determining their toxicity profiles at the cell-level and organ level), in order to accelerate their clinical translation.

7.5 Sub-cellular targeting

It is often assumed that mediating cell cytosolic internalization is adequate to ensure the interaction of drug molecules with their final subcellular targets *via* simple diffusion and random interactions. However, it has become increasingly evident that such an assumption does not likely hold true.[401–407] In addition to the presence of the cytoskeletal network and various dispersed organelles, the cytoplasm contains a large amount of dissolved macromolecules, with a concentration between 50 and 400 g L^{-1}.[408, 409] Consequently, transport or diffusion events in such a crowded solution cannot be expected to be the same as those in buffer solutions. In the case of a drug molecule with no defined specificity for a particular organelle, the molecule would need to have sufficiently long metabolic stability to allow for random interactions with the organelle. In the case of molecules with a stronger affinity for a non-target subcellular compartment, there exists a greater need for the ability to control subcellular disposition. In the case of drug molecules that are recognized by the drug efflux pump (*e.g.* P-glycoprotein), the design of NPs that can be internalized by endocytosis and thus release their active drugs inside subcellular organelles can be used to overcome multidrug resistance in cancer cells.[410, 411] In addition, increasing attention has been focused on the pathological disorders of subcellular organelles (*e.g.* endosomes and lysosomes), which could potentially benefit from therapies targeting these pathways.[412] For example, endosomal abnormalities in neurons have been associated with the etiology of Alzheimer's disease.[413] Lysosomes and in particular lysosomal hydrolases have been associated with several aspects of malignant transformation, including the loss of cell

growth control, altered regulation of cell death, and acquisition of chemo-resistance and of metastatic potential.[414] Endosomes and lysosomes have thus been proposed as potential target organelles for the chemotherapy of Alzheimer's disease and cancers. Subsequently, focus has shifted towards subcellular drug targeting, that is, directing therapeutic agents to an individual organelle and is now becoming a new frontier for the development of therapeutic NPs.[415]

Two major approaches have been applied in the design of NPs with the potential for subcellular targeting. The first is based on attaching subcellular targeting ligands on the surface of NPs to redirect their accumulation to the desired compartment. The second is based on the inherent predisposition of the NPs for a particular compartment, which can be influenced at the preparation stage of NPs by using components that have a strong affinity for a subcellular compartment, and tuning the NP aspect ratio and size.

The type of targeting ligands displayed by NPs will determine the deposition location of these agents inside the cells. Well-characterized endocytic targeting ligands potentially useful for NP-mediated drug delivery are folic acid, low-density lipoprotein, cholera toxin B, mannose-6-phosphate, Tf, riboflavin, the tripeptide RGD, ICAM-1 antibody and nicotinic acid, as reviewed elsewhere.[412] The cellular internalization mechanisms utilized by these ligands involve clathrin-dependent receptor mediated endocytosis, caveolin-assisted endocytosis, lipid raftassociated endocytosis and cell adhesionmolecule (CAM)-directed cellular uptake.[412, 416, 417]

Tfs,[418] a family of large non-heme iron-binding glycoproteins, are the most widely used endocytic targeting ligands for the functionalization of NP drug delivery systems. Iron-loaded transferrin binds to a specific cell-surface receptor (TfR1) and is taken up *via* clathrin-coated pits. The TfR complex is routed into the endosomal compartment, avoiding lysosomal digestion. This is an important feature of TfR1 for NP delivery, as normally glycoproteins taken up by means of receptor-mediated endocytosis are destined eventually to fuse with lysosomes. Encapsulation of Dox into liposomes bearing Tf on the distal end of liposomal PEG chains resulted in significantly increased Dox uptake into glioma cells, which are known to overexpress the TfRs.[419] Tf modification of Dox-loaded palmitoylated glycol chitosan (GCP) vesicles resulted in higher uptake and increased cytotoxicity as compared with GCP doxorubicin alone.[420]

Intercellular adhesion molecule (ICAM)-1, a glycoprotein expressed on diverse cell types, is upregulated and functionally involved in inflammation, which is a hallmark of many lysosomal disorders.[421] Recombinant human acid sphingomyelinase (ASM) enzyme, deficient in types A and B Niemann-Pick disease, was loaded into NPs coated with anti-ICAM antibody. Anti-ICAM/ASM NPs were found to enter cells by means of CAM-mediated endocytosis and traffic to lysosomes. The delivered enzyme displayed stable activity and alleviated lysosomal lipid accumulation, suggesting that NPs targeted to ICAM-1 bypassed defunct pathways and could improve the efficacy of enzyme replacement therapy for lysosomal disorders, such as Niemann-Pick disease.[421] A mitochondrial leader peptide (MLP), derived from the nucleocytosol-expressed but mitochondria-localized ornithine transcarbamylase, was used to render polyethylenimine (PEI)NPs mitochondriotropic.[422] Lee *et al.*[422] conjugated the mitochondrial leader peptide to PEI NPs by means of a disulfide bond and confirmed the complex formation of PEI–MLP with DNA by a gel retardation assay. *In vitro* delivery tests of rhodamine-labelled DNA into living cells demonstrated that PEI–MLP/DNA complexes were localized at mitochondrial sites. The data suggested that PEI–MLP can deliver DNA to the mitochondrial sites and may be useful for the development of direct mitochondrial gene therapy.

In an elegant recent study, Murakami *et al.* investigated the real-time subcellular fate of polymeric micelles formed from (1,2-diaminocyclohexane) platinum(II) (DACHPt/m), the parent complex of oxaliplatin, in tumour tissues using fluorescence aided based assessment of their kinetic stability (Fig. 17).[423] They were able to show potent antitumour activity for their system and to prove that their designed polymeric micelles were able to overcome Pt resistance both *in vitro* and *in vivo*. The extravasation of DACHP/m NP was observed from blood vessels into tumours in addition to polymer dissociation intracellularly. It was hypothesized that these polymeric NPs selectively dissociated in the late endosomes and facilitated Pt drug delivery to the nucleus relative to free oxaliplatin. The authors proposed that this outcome is most likely due to circumvention of the cytoplasmic detoxification systems of metallothionein and methionine synthase. By developing oxaliplatin resistance in various cell lines, they were able to test this hypothesis as in each case the drug loaded micelles exhibited higher toxicities than the free oxaliplatin. This study suggests that therapeutic NPs have enormous potential in both intracellular targeting *via* compartmentalization, and drug delivery in an efficacious manner.

7.6 Stimuli responsive and surface switching NPs

An emerging method for targeting drugs to disease areas is to exploit the local changes that occur due to disease pathology and use these changes as triggers to improve targeting. This is accomplished by developing stimuli-responsive materials that change their physicochemical or drug-release properties upon encountering specific environmental cues, potentially leading to increased drug delivery to diseased tissues.[424–426] These methods of drug targeting have grown out of both a greater understanding of the local changes that occur in different disease conditions and through a greater ability to design materials that have a dynamic range that is physiologically relevant for the specific disease application. These stimuli can be classified as extracellular, intracellular, or both. Extracellular conditions that may be altered in disease typically include decreased pH, such as in certain solid tumours, inflammation, and infections, and the presence of certain enzymes, such as the clotting cascade.[426] However, extracellular conditions can also be artificially manipulated such as by applying local heating, ultrasound, or near infrared light to trigger an effect.[427, 428] Intracellular conditions that can be exploited include low pH, presence of certain unique enzymes, and the reducing environment of endolysosomes.[429, 430]

Stimuli-responsive materials used for drug delivery either trigger a burst of drug release or increase the rate of drug release in response to a given stimuli.[431–433] In addition, it may be possible to improve NP targeting to sites of disease by causing changes in NP surface properties at sites of disease.[434]

These strategies can be applied broadly across different types of NPs and are not limited to polymeric NPs. For example, liposomes that have multiple targeting functions that are unveiled sequentially have been developed to respond to extracellular low pH to improve intracellular delivery.[435, 436] In a recent study, Poon *et al.* developed polylysine-coated quantum dots *via* layer-by-layer (LbL) assembly.[197] The neutral layers on the surface of these NPs were designed to shed in response to the acidic tumour environment to reveal a highly charged surface that facilitated uptake by tumour cells. The results of this study demonstrated the potential of LbL NPs for both tumour targeting and cancer cell targeting.

Stimuli-responsive polymeric micelle NPs based on block copolymers have been developed extensively by Bae *et al.*[57] A few illustrative examples include poly(L-histidine)-*b*-polyethylene glycol/poly(L-lactide)-*b*-polyethylene glycol mixed micelles that dissolve at low pH, leading to low-pH-targeted drug release.[437] The pH at which these micelles dissolved could be tuned by changing the ratio of poly(L-lactide)-*b*-polyethylene glycol to poly(L-histidine)-*b*-polyethylene glycol, demonstrating the potential to apply this drug

deliverymethod to a variety of different tumours or specific areas within tumours based on the local acidity.[437] Another example is a TAT-peptide-modified triblock copolymer of poly(L-lactic acid)-*b*-poly(ethylene glycol)-*b*-poly-(L-histidine)-TAT mixed with poly(L-histidine)-*b*-poly(ethylene glycol) to yield micelles that present TAT-peptide on encountering acidity for targeted internalization.[438] In this case, pH-sensitive TAT-peptide exposure on the micelle surface is believed to be significant for tumour cell-specific internalization, as TAT-peptide is known to interact with various different cell membranes, such as the BBB.[439]

Other stimuli-responsive polymeric NPs have been developed, a targeted, environment-responsive polymeric nanocapsule based on cucurbit[6]uril, was also designed to collapse in reducing environments, such as inside cells, to trigger burst drug release.[159] Additionally, temperature-sensitive NPs based on poly(*N*-isopropylacrylamide)- *b*-poly(butyl methacrylate) were designed to release drugs rapidly at temperatures above the lower critical solution temperature (LCST) of the poly(*N*-isopropylacrylamide) segment (32 °C).[440]

These and other methods of using stimuli to improve drug targeting are likely to see continued development, particularly in instances where a high level of differentiation between pathologic and physiologic conditions exists.

8. Opportunities and challenges for targeted NPs

Oncology is one area where nanomedicine products are set tomake the most impact, where cell and tissue targeting approaches can be used to efficiently deliver cytotoxic and molecularly targeted drugs to cancer cells. Most cancer drugs today are administered either intravenously or orally and become systemically distributed without preferential partitioning to cancer tissue. The widespread biodistribution of cancer drugs results in both therapeutic anti-cancer effects as well as off target adverse effects on healthy and proliferating non-cancer cells. The ability to target therapeutics in a more controlled and specific manner is the primary goal of developing targeted NPs. The molecularly targeted cancer drugs typically modulate signalling pathways that are aberrantly activated in cancer cells. These drugs are most commonly: (i) administered orally, (ii) dosed daily, (iii) interact with their targets reversibly, and (iv) are limited in their efficacy as single agents due to factors such as network robustness, redundancy, crosstalk and emergence of resistance. Polymeric NP technologies may enable the co-delivery of multiple molecularly targeted drugs, creating an integrative pharmacologic effect among distinct drugs, thereby modulating multiple pathways that may translate into more prolonged and efficacious anti-cancer therapies.

While the potential advantages of delivering molecularly targeted drugs *via* targeted polymericNPs is relatively clear, one potential challenge arises due to the fact that polymeric NPs are most commonly administered intravenously. This necessitates efficient NP capabilities for encapsulating sufficiently high drug concentrations in order to ensure a constant release of drugs—in particular, between infrequent intravenous (IV) dosing regimens.

Numerous works have demonstrated the effectiveness of targeted NPs in becoming internalized into cells and delivering therapeutic and imaging agents in a highly specific manner (see Table 2). For example, targeted polymeric NPs were shown to elicit marked increase in intracellular uptake by prostate cancer cells in comparison to non-targeted NPs *in vitro*.[195, 282, 351, 441, 442] Furthermore, the careful tuning of targeting ligands density and chemical properties on the surface of NPs was shown to further enhance the intracellular uptake of polymeric NPs in targeted cells.[218, 266] Ultimately a number of parameters need to be investigated for successful design of targeted NPs, which include optimization of NP biophysicochemical properties and the demonstration of the efficacy of targeted NPs in a

clinical setting on their impact on patient outcomes. Aside from identification of optimal ligands and ligand targets suitable for highly selective NP targeting, a whole host of other practical challenges in the development of targeted therapeutic NPs should also be considered including: (1) the use of biocompatible, biodegradable/bioeliminable materials, (2) the use of simple, robust and reproducible bioconjugation chemistries for the attachment of precursors and targeting ligands, (3) facile NP assemblies that avoid multi-step NP preparation and purification steps, (4) optimization of NP biophysicochemical properties to achieve optimal drug load/release, long circulation half-life, suitable biodistribution, differential target tissue accumulation, efficacious target tissue drug concentration and drug exposure kinetics, (5) validation of NP stability and predictable shelf-life; and, (6) development or adaptation of scalable processes and units of operations amenable to the manufacturing of large quantities of targeted NPs for clinical development and commercialization.

Recently interest has developed on how the properties of size, shape, surface area, roughness, porosity, surface functional groups, ligands, surface defects, hydrophobicity and hydrophilicity (Fig. 12) collectively influence NP behaviour at the ‘nano-bio’ interface, as this understanding is key to improved NP design.[353] Indeed, the value of tailoring these parameters with the purpose of minimizing toxicity, unfavourable interactions with the immune system, rapid renal clearance, and accumulation in organs such as the liver and spleen is beginning to be more systematically recognized and increasingly adopted.[443]

Targeted NPs can lead to nanomedicines being specifically retained in tissues and/or cells, resulting in higher dose and duration of drug exposure within the target tissue. However, ultimately the question remains as to whether targeted NPs demonstrate marked improvement in clinical outcomes, which need to be demonstrated through well executed larger clinical trials. Beyond the regulatory requirements of demonstrating safety, efficacy, quality and cost-effectiveness, further challenges of each targeted NP technology need to be investigated on a case-by-case basis and these challenges must be met in order to harness their tremendous potential as a new class of targeted therapeutics.

9. Conclusions and outlook

Polymeric NPs have shown tremendous therapeutic potential at both research and clinical levels. Coupled with the fast pace of development in nanotechnology and our deeper understanding of biophysicochemical parameters that govern NP behaviour in biological systems, then it is not over reaching to expect NP technologies to create revolutionary therapies for a myriad of medical problems. This optimism is justified given our improved knowledge of disease pathways, better manipulation, characterization and control of matter at the nanoscale, and proven clinical outcomes so far. We are now in a good position to no longer look at targeted polymeric NPs as merely nanosized drug delivery vehicles, but to view them in a new light—as a new class of therapeutics that impact treatment efficacies beyond that of the action of the drug itself. In particular, this concept is now being realized with respect to the modes of drug release at active sites both extra and intracellularly, which is governed by the therapeutic NP capabilities of undergoing endocytosis assisted uptake, bypassing of MDR pathways and targeting subcellular compartments.

We have confidence that with a well characterized system including: safe, effective, and specific targeting ligands, biocompatible, biodegradable and bioeliminable materials, and appropriate choice of therapeutics and disease models, targeted polymeric NPs could yield more effective treatments for a myriad of important human diseases. The exciting developments that are producing more “sensitive” NPs capable of triggered release of drugs at active sites under environmental cues further strengthen the therapeutic NP arsenal.

Although targeted NPs have been slow to enter the clinic their potential as an entirely new class of therapeutics, remains tremendous.

A considerable amount of research and development is necessary from the proof-of-principle stage of developing novel targeted nanomedicines to their bench-to-bedside translation, and in particular, the multifunctionality and complexity of some targeted polymeric NPs should be investigated on a case-by-case basis. The processes for the engineering of targeted NPs must be carefully developed and controlled in order to facilitate reproducible and scalable NP production. The development of complementary technologies such as the identification of biochemically stable non-immunogenic ligands with higher affinities and specificity for clinically relevant targets is of potential importance. Selective adaptation of NP technologies early during drug discovery may also result in the development of improved drugs beyond what is achievable by medicinal chemistry alone. For example NP technologies can protect drugs from rapid metabolism and inactivation; improve drug solubility, PK, BD, and target tissue exposure. All of these properties are typically optimized by medicinal chemistry efforts and often occur at the expense of other desirable properties including for example, drug potency or specificity. The combination of medicinal chemistry and NP engineering early on during drug discovery holds enormous promise, and provides additional degrees of freedom to medicinal chemistry efforts for creating optimally developed targeted NP therapeutics. With continued infusion of capital from the public and private sectors toward the research and development in the area of nanomedicine we believe that targeted polymeric NP technologies will emerge as an important class of therapeutics in the next 20 years with broad therapeutic impact, analogous to the evolution and impact of mAb technologies over the last few decades. There is still "plenty of room at the bottom".[444]

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Aleks Radovic-Moreno completed his B.S. with Honors in Chemical Engineering at the Pennsylvania State University in 2005. He is currently a PhD Candidate and NSF fellow in the Harvard-MIT Division of Health Sciences & Technology and a member of the Langer Lab at MIT. His work has focused on developing targeted polymeric nanoparticles for treating cancer in addition to bacterial infections. More specifically, he has designed various new drug encapsulation and delivery strategies, including methods to improve drug targeting using aptamer-functionalized or surface charge-switching nanoparticles.

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References

1. LaVan DA, McGuire T, Langer R. Nat. Biotechnol. 2003; 21:1184–1191. [PubMed: 14520404]
2. Langer R. Science. 1990; 249:1527–1533. [PubMed: 2218494]
3. Shi J, Xiao Z, Kamaly N, Farokhzad OC. Acc. Chem. Res. 2011; 44:1123–1134. [PubMed: 21692448]
4. Davis ME, Chen ZG, Shin DM. Nat. Rev. Drug Discovery. 2008; 7:771–782.
5. Farokhzad OC. Expert Opin. Drug Delivery. 2008; 5:927–929.
6. Bangham AD, Standish MM, Watkins JC. J. Mol. Biol. 1965; 13:238–252. [PubMed: 5859039]
7. Farokhzad OC, Langer R. ACS Nano. 2009; 3:16–20. [PubMed: 19206243]
8. Wagner V, Dullaart A, Bock AK, Zweck A. Nat. Biotechnol. 2006; 24:1211–1217. [PubMed: 17033654]
9. Bangham AD, Horne RW. J. Mol. Biol. 1964; 8:660–668. [PubMed: 14187392]
10. Langer R, Folkman J. Nature. 1976; 263:797–800. [PubMed: 995197]
11. Buhleier E, Wehner W, Vogtle F. Synthesis. 1978:155–158.
12. Torchilin VP. Immuno Methods. 1994; 4:244–258.
13. Gabizon A, Shmeeda H, Barenholz Y. Clin. Pharmacokinet. 2003; 42:419–436. [PubMed: 12739982]
14. Gref R, Minamitake Y, Peracchia MT, Trubetskoy V, Torchilin V, Langer R. Science. 1994; 263:1600–1603. [PubMed: 8128245]
15. Ros PR, Freeny PC, Harms SE, Seltzer SE, Davis PL, Chan TW, Stillman AE, Muroff LR, Runge VM, Nissenbaum MA, et al. Radiology. 1995; 196:481–488. [PubMed: 7617864]
16. Gradishar WJ, Tjulandin S, Davidson N, Shaw H, Desai N, Bhar P, Hawkins M, O'Shaughnessy J. J. Clin. Oncol. 2005; 23:7794–7803. [PubMed: 16172456]
17. Kim TY, Kim DW, Chung JY, Shin SG, Kim SC, Heo DS, Kim NK, Bang YJ. Clin. Cancer Res. 2004; 10:3708–3716. [PubMed: 15173077]
18. Davis ME. Mol. Pharmaceutics. 2009; 6:659–668.
19. http://clinicaltrials.gov/ct2/show/NCT01300533
20. http://www.clinicaltrials.gov/ct2/show/NCT01478893
21. Allen TM, Chonn A. FEBS Lett. 1987; 223:42–46. [PubMed: 3666140]
22. Leserman LD, Barbet J, Kourilsky F, Weinstein JN. Nature. 1980; 288:602–604. [PubMed: 7442804]
23. Heath TD, Fraley RT, Papahdjopoulos D. Science. 1980; 210:539–541. [PubMed: 7423203]

24. Burgess P, Hutt PB, Farokhzad OC, Langer R, Minick S, Zale S. Nat. Biotechnol. 2010; 28:1267–1270. [PubMed: 21139609]
25. MacDonald ML, Lamerdin J, Owens S, Keon BH, Bilter GK, Shang Z, Huang Z, Yu H, Dias J, Minami T, Michnick SW, Westwick JK. Nat. Chem. Biol. 2006; 2:329–337. [PubMed: 16680159]
26. McNeil SE. J. Leukocyte Biol. 2005; 78:585–594. [PubMed: 15923216]
27. http://clinicaltrials.gov/ct2/show/NCT00689065
28. Davis ME. Mol. Pharmaceutics. 2009; 6:659–668.
29. Sankhala KK, Mita AC, Adinin R, Wood L, Beeram M, Bullock S, Yamagata N, Matsuno K, Fujisawa T, Phan A. J. Clin. Oncol. 2009; 27:S2535.
30. Matsumura Y, Gotoh M, Muro K, Yamada Y, Shirao K, Shimada Y, Okuwa M, Matsumoto S, Miyata Y, Ohkura H, Chin K, Baba S, Yama T, Kannami A, Takamatsu Y, Ito K, Takahashi K. Ann. Oncol. 2004; 15:517–525. [PubMed: 14998859]
31. Nemunaitis J, Senzer N, Bedell C, Nunan R, Sleer L, Chang E. Mol. Ther. 2009; 17:S226.
32. Barreto JA, O'Malley W, Kubeil M, Graham B, Stephan H, Spiccia L. Adv. Mater. 2011; 23:H18–40. [PubMed: 21433100]
33. Langer R, Tirrell DA. Nature. 2004; 428:487–492. [PubMed: 15057821]
34. Langer R. Nature. 1998; 392:5–10. [PubMed: 9579855]
35. Udhrain A, Skubitz KM, Northfelt DW. Int. J. Nanomedicine. 2007; 2:345–352. [PubMed: 18019833]
36. Campos SM, Penson RT, Mays AR, Berkowitz RS, Fuller AF, Goodman A, Matulonis UA, Muzikansky A, Seiden MV. Gynecol. Oncol. 2001; 81:206–212. [PubMed: 11330951]
37. Pinto-Alphandary H, Andremont A, Couvreur P. Int. J. Antimicrob. Agents. 2000; 13:155–168. [PubMed: 10724019]
38. Fattal E, Rojas J, Roblot-Treupel L, Andremont A, Couvreur P. J. Microencapsulation. 1991; 8:29–36. [PubMed: 1880689]
39. Montana M, Ducros C, Verhaeghe P, Terme T, Vanelle P, Rathelot P. J. Chemother. 2011; 23:59–66. [PubMed: 21571619]
40. Gradishar WJ, Tjulandin S, Davidson N, Shaw H, Desai N, Bhar P, Hawkins M, O'Shaughnessy J. J. Clin. Oncol. 2005; 23:7794–7803. [PubMed: 16172456]
41. Sparreboom A, Scripture CD, Trieu V, Williams PJ, De TP, Yang A, Beals B, Figg WD, Hawkins M, Desai N. Clin. Cancer Res. 2005; 11:4136–4143. [PubMed: 15930349]
42. Lee KS, Chung HC, Im SA, Park YH, Kim CS, Kim SB, Rha SY, Lee MY, Ro J. Breast Cancer Res. Treat. 2008; 108:241–250. [PubMed: 17476588]
43. Park SR, Oh DY, Kim DW, Kim TY, Heo DS, Bang YJ, Kim NK, Kang WK, Kim HT, Im SA, Suh JH, Kim HK. Oncol. Rep. 2004; 12:1059–1064. [PubMed: 15492793]
44. Marshall J. Nature News. 2011
45. Chan JM, Valencia PM, Zhang L, Langer R, Farokhzad OC. Methods Mol. Biol. 2010; 624:163–175. [PubMed: 20217595]
46. Kolishetti N, Dhar S, Valencia PM, Lin LQ, Karnik R, Lippard SJ, Langer R, Farokhzad OC. Proc. Natl. Acad. Sci. U. S. A. 2010; 107:17939–17944. [PubMed: 20921363]
47. Hearn EM, Patel DR, Lepore BW, Indic M, van den Berg B. Nature. 2009; 458:367–U134. [PubMed: 19182779]
48. Ferrari M. Nat. Rev. Cancer. 2005; 5:161–171. [PubMed: 15738981]
49. Gottesman MM, Fojo T, Bates SE. Nat. Rev. Cancer. 2002; 2:48–58. [PubMed: 11902585]
50. Seow WY, Xue JM, Yang YY. Biomaterials. 2007; 28:1730–1740. [PubMed: 17182095]
51. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nat. Nanotechnol. 2007; 2:751–760. [PubMed: 18654426]
52. Lee ES, Na K, Bae YH. J. Controlled Release. 2005; 103:405–418.
53. Sahoo SK, Ma W, Labhasetwar V. Int. J. Cancer. 2004; 112:335–340. [PubMed: 15352049]
54. Wang X, Li J, Wang Y, Koenig L, Gjyrezi A, Giannakakou P, Shin EH, Tighiouart M, Chen ZG, Nie S, Shin DM. ACS Nano. 2011; 5:6184–6194. [PubMed: 21728341]
55. York AW, Kirkland SE, McCormick CL. Adv. Drug Delivery Rev. 2008; 60:1018–1036.

56. Dhal PK, Polomoscanik SC, Avila LZ, Holmes-Farley SR, Miller RJ. Adv. Drug Delivery Rev. 2009; 61:1121–1130.
57. Bae Y, Kataoka K. Adv. Drug Delivery Rev. 2009; 61:768–784.
58. Drummond DC, Meyer O, Hong K, Kirpotin DB, Papahadjopoulos D. Pharmacol. Rev. 1999; 51:691–743. [PubMed: 10581328]
59. Caldorera-Moore ME, Liechty WB, Peppas NA. Acc. Chem. Res. 2011; 44:1061–1070. [PubMed: 21932809]
60. Hoffman AS. J. Controlled Release. 2008; 132:153–163.
61. Kleber JW, Nash JF, Lee CC. J. Pharm. Sci. 1964; 53:1519–1521. [PubMed: 14255135]
62. Farokhzad OC, Langer R. Adv. Drug Delivery Rev. 2006; 58:1456–1459.
63. Acharya S, Sahoo SK. Adv. Drug Delivery Rev. 2011; 63:170–183.
64. Shive MS, Anderson JM. Adv. Drug Delivery Rev. 1997; 28:5–24.
65. Langer R. Pharmacol. Ther. 1983; 21:35–51. [PubMed: 6353438]
66. West CP, Lumsden MA, Lawson S, Williamson J, Baird DT. Fertil. Steril. 1987; 48:45–51. [PubMed: 2954863]
67. Sethi R, Sanfilippo N. Clin. Interv. Aging. 2009; 4:259–267. [PubMed: 19554097]
68. Lin SH, Kleinberg LR. Expert Rev. Anti-Infect. Ther. 2008; 8:343–359.
69. Johnson LR, Stoller NH. Compend. Contin. Educ. Dent. 1999; 20:19–25. quiz 35. [PubMed: 11908360]
70. Anthony L, Freda PU. Curr. Med. Res. Opin. 2009; 25:2989–2999. [PubMed: 19842996]
71. Crawford ED, Phillips JM. Cancer Manage. Res. 2011; 3:201–209.
72. Kreuter J. Pharm. Acta Helv. 1978; 53:33–39. [PubMed: 353818]
73. Maeda H. Bioconjugate Chem. 2010; 21:797–802.
74. Matsumura Y, Maeda H. Cancer Res. 1986; 46:6387–6392. [PubMed: 2946403]
75. Maeda H, Bharate GY, Daruwalla J. Eur. J. Pharm. Biopharm. 2009; 71:409–419. [PubMed: 19070661]
76. Saha RN, Vasanthakumar S, Bende G, Snehalatha M. Mol. Membr. Biol. 2010; 27:215–231. [PubMed: 20939772]
77. Torchilin VP. Handb. Exp. Pharmacol. 2010:3–53. [PubMed: 20217525]
78. Carmeliet P, Jain RK. Nature. 2000; 407:249–257. [PubMed: 11001068]
79. Danquah MK, Zhang XA, Mahato RI. Adv. Drug Delivery Rev. 2011; 63:623–639.
80. Yuan F, Dellian M, Fukumura D, Leunig M, Berk DA, Torchilin VP, Jain RK. Cancer Res. 1995; 55:3752–3756. [PubMed: 7641188]
81. Suzuki M, Hori K, Abe I, Saito S, Sato H. J. Natl. Cancer Inst. 1981; 67:663–669. [PubMed: 6944536]
82. Fang J, Nakamura H, Maeda H. Adv. Drug Delivery Rev. 2011; 63:136–151.
83. Mulder WJ, Douma K, Koning GA, van Zandvoort MA, Lutgens E, Daemen MJ, Nicolay K, Strijkers GJ. Magn. Reson. Med. 2006; 55:1170–1174. [PubMed: 16598732]
84. Nagamitsu A, Greish K, Maeda H. Jpn. J. Clin. Oncol. 2009; 39:756–766. [PubMed: 19596662]
85. Chrastina A, Massey KA, Schnitzer JE. Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol. 2011; 3:421–437. [PubMed: 21538941]
86. Hobbs SK, Monsky WL, Yuan F, Roberts WG, Griffith L, Torchilin VP, Jain RK. Proc. Natl. Acad. Sci. U. S. A. 1998; 95:4607–4612. [PubMed: 9539785]
87. Jain RK. Adv. Drug Delivery Rev. 2001; 46:149–168.
88. Monsky WL, Fukumura D, Gohongi T, Ancukiewcz M, Weich HA, Torchilin VP, Yuan F, Jain RK. Cancer Res. 1999; 59:4129–4135. [PubMed: 10463618]
89. Kano MR, Bae Y, Iwata C, Morishita Y, Yashiro M, Oka M, Fujii T, Komuro A, Kiyono K, Kaminishi M, Hirakawa K, Ouchi Y, Nishiyama N, Kataoka K, Miyazono K. Proc. Natl. Acad. Sci. U. S. A. 2007; 104:3460–3465. [PubMed: 17307870]
90. Hatakeyama H, Akita H, Harashima H. Adv. Drug Delivery Rev. 2011; 63:152–160.
91. Romberg B, Hennink WE, Storm G. Pharm. Res. 2008; 25:55–71. [PubMed: 17551809]

92. Horton JK, Houghton PJ, Houghton JA. Biochem. Pharmacol. 1988; 37:3995–4000. [PubMed: 3190743]
93. Jordan MA, Wendell K, Gardiner S, Derry WB, Copp H, Wilson L. Cancer Res. 1996; 56:816–825. [PubMed: 8631019]
94. Kuh HJ, Jang SH, Wientjes MG, Weaver JR, Au JL. J. Pharmacol. Exp. Ther. 1999; 290:871–880. [PubMed: 10411604]
95. Zamboni WC, Strychor S, Joseph E, Parise RA, Egorin MJ, Eiseman JL. Cancer Chemother. Pharmacol. 2008; 62:417–426. [PubMed: 17957368]
96. Bissery MC. Eur. J. Cancer. 1995; 31(Suppl 4):S1–S6. [PubMed: 7577097]
97. Ishida T, Ichihara M, Wang X, Yamamoto K, Kimura J, Majima E, Kiwada H. J. Controlled Release. 2006; 112:15–25.
98. Ishida T, Kiwada H. Yakugaku Zasshi. 2008; 128:233–243. [PubMed: 18239370]
99. Valle JW, Armstrong A, Newman C, Alakhov V, Pietrzynski G, Brewer J, Campbell S, Corrie P, Rowinsky EK, Ranson M. Invest. New Drugs. 2011; 29:1029–1037. [PubMed: 20179989]
100. Matsumura Y, Hamaguchi T, Ura T, Muro K, Yamada Y, Shimada Y, Shirao K, Okusaka T, Ueno H, Ikeda M, Watanabe N. Br. J. Cancer. 2004; 91:1775–1781. [PubMed: 15477860]
101. Adams ML, Lavasanifar A, Kwon GS. J. Pharm. Sci. 2003; 92:1343–1355. [PubMed: 12820139]
102. Danson S, Ferry D, Alakhov V, Margison J, Kerr D, Jowle D, Brampton M, Halbert G, Ranson M. Br. J. Cancer. 2004; 90:2085–2091. [PubMed: 15150584]
103. Oerlemans C, Bult W, Bos M, Storm G, Nijsen JF, Hennink WE. Pharm. Res. 2010; 27:2569–2589. [PubMed: 20725771]
104. Sutton D, Nasongkla N, Blanco E, Gao J. Pharm. Res. 2007; 24:1029–1046. [PubMed: 17385025]
105. Boddy AV, Plummer ER, Todd R, Sludden J, Griffin M, Robson L, Cassidy J, Bissett D, Bernareggi A, Verrill MW, Calvert AH. Clin. Cancer Res. 2005; 11:7834–7840. [PubMed: 16278406]
106. Galic VL, Herzog TJ, Wright JD, Lewin SN. Expert Opin. Invest. Drugs. 2011; 20:813–821.
107. Schluep T, Hwang J, Cheng J, Heidel JD, Bartlett DW, Hollister B, Davis ME. Clin. Cancer Res. 2006; 12:1606–1614. [PubMed: 16533788]
108. Jokerst JV, Lobovkina T, Zare RN, Gambhir SS. Nanomedicine. 2011; 6:715–728. [PubMed: 21718180]
109. Knop K, Hoogenboom R, Fischer D, Schubert US. Angew. Chem., Int. Ed. 2010; 49:6288–6308.
110. Harris JM, Chess RB. Nat. Rev. Drug Discovery. 2003; 2:214–221.
111. Kaminskas LM, McLeod VM, Porter CJ, Boyd BJ. J. Pharm. Sci. 2011; 100:5069–5077. [PubMed: 21721002]
112. Tagami T, Uehara Y, Moriyoshi N, Ishida T, Kiwada H. J. Controlled Release. 2011; 151:149–154.
113. Koide H, Asai T, Hatanaka K, Urakami T, Ishii T, Kenjo E, Nishihara M, Yokoyama M, Ishida T, Kiwada H, Oku N. Int. J. Pharm. 2008; 362:197–200. [PubMed: 18586076]
114. Lu W, Wan J, She Z, Jiang X. J. Controlled Release. 2007; 118:38–53.
115. Ma H, Shiraishi K, Minowa T, Kawano K, Yokoyama M, Hattori Y, Maitani Y. Pharm. Res. 2010; 27:296–302. [PubMed: 20035375]
116. Xu H, Wang KQ, Deng YH, Chen da W. Biomaterials. 2010; 31:4757–4763. [PubMed: 20303164]
117. Hamad I, Hunter AC, Szebeni J, Moghimi SM. Mol. Immunol. 2008; 46:225–232. [PubMed: 18849076]
118. Hamad I, Al-Hanbali O, Hunter AC, Rutt KJ, Andresen TL, Moghimi SM. ACS Nano. 2010; 4:6629–6638. [PubMed: 21028845]
119. Jiang S, Cao Z. Advanced materials (Deerfield Beach, Fla). 2010; 22:920–932.
120. Vaisocherova H, Yang W, Zhang Z, Cao Z, Cheng G, Piliarik M, Homola J, Jiang S. Anal. Chem. 2008; 80:7894–7901. [PubMed: 18808152]
121. Banerjee I, Pangule RC, Kane RS. Adv. Mater. 2010; 23:690–718. [PubMed: 20886559]

122. Yu B, Tai HC, Xue W, Lee LJ, Lee RJ. Mol. Membr. Biol. 2010; 27:286–298. [PubMed: 21028937]
123. Kolhatkar R, Lote A, Khambati H. Curr. Drug Discovery Technol. 2011; 8:197–206.
124. Farokhzad OC, Cheng J, Teply BA, Sherifi I, Jon S, Kantoff PW, Richie JP, Langer R. Proc. Natl. Acad. Sci. U. S. A. 2006; 103:6315–6320. [PubMed: 16606824]
125. Lammers T, Hennink WE, Storm G. Br. J. Cancer. 2008; 99:392–397. [PubMed: 18648371]
126. Warenius HM, Galfre G, Bleehen NM, Milstein C. Eur. J. Cancer Clin. Oncol. 1981; 17:1009–1015. [PubMed: 7198983]
127. www.antibodysociety.org/news/approved_mabs.php
128. Hooks MA, Wade andW. J.Millikan, Jr. CS. Pharmacotherapy. 1991; 11:26–37. [PubMed: 1902291]
129. James JS, Dubs G. AIDS Treat News. 1997:2–3.
130. Albanell J, Baselga J. Drugs Today (Barc). 1999; 35:931–946. [PubMed: 12973420]
131. Graham MM, Menda Y. J. Nucl. Med. 2011; 52(Suppl 2):56S–63S. [PubMed: 22144556]
132. Sudimack J, Lee RJ. Adv. Drug Delivery Rev. 2000; 41:147–162.
133. Qian ZM, Li H, Sun H, Ho K. Pharmacol. Rev. 2002; 54:561–587. [PubMed: 12429868]
134. Allen TM. Nat. Rev. Cancer. 2002; 2:750–763. [PubMed: 12360278]
135. Sugano M, Egilmez NK, Yokota SJ, Chen FA, Harding J, Huang SK, Bankert RB. Cancer Res. 2000; 60:6942–6949. [PubMed: 11156394]
136. Bareford LM, Swaan PW. Adv. Drug Delivery Rev. 2007; 59:748–758.
137. Whitehead KA, Langer R, Anderson DG. Nat. Rev. Drug Discovery. 2009; 8:129–138.
138. Schroeder A, Levins CG, Cortez C, Langer R, Anderson DG. J. Intern. Med. 2010; 267:9–21. [PubMed: 20059641]
139. Varkouhi AK, Scholte M, Storm G, Haisma HJ. J. Controlled Release. 2011; 151:220–228.
140. Alexis F, Basto P, Levy-Nissenbaum E, Radovic-Moreno AF, Zhang L, Pridgen E, Wang AZ, Marein SL, Westerhof K, Molnar LK, Farokhzad OC. ChemMedChem. 2008; 3:1839–1843. [PubMed: 19012296]
141. Kirpotin DB, Drummond DC, Shao Y, Shalaby MR, Hong K, Nielsen UB, Marks JD, Benz CC, Park JW. Cancer Res. 2006; 66:6732–6740. [PubMed: 16818648]
142. Bartlett DW, Su H, Hildebrandt IJ, Weber WA, Davis ME. Proc. Natl. Acad. Sci. U. S. A. 2007; 104:15549–15554. [PubMed: 17875985]
143. Pun SH, Tack F, Bellocq NC, Cheng J, Grubbs BH, Jensen GS, Davis ME, Brewster M, Janicot M, Janssens B, Floren W, Bakker A. Cancer Biol. Ther. 2004; 3:641–650. [PubMed: 15136766]
144. Hu-Lieskovan S, Heidel JD, Bartlett DW, Davis ME, Triche TJ. Cancer Res. 2005; 65:8984–8992. [PubMed: 16204072]
145. Pirollo KF, Chang EH. Trends Biotechnol. 2008; 26:552–558. [PubMed: 18722682]
146. Samanta S, Sistla R, Chaudhuri A. Biomaterials. 2010; 31:1787–1797. [PubMed: 19889452]
147. Chan JM, Zhang L, Tong R, Ghosh D, Gao W, Liao G, Yuet KP, Gray D, Rhee JW, Cheng J, Golomb G, Libby P, Langer R, Farokhzad OC. Proc. Natl. Acad. Sci. U. S. A. 2010; 107:2213–2218. [PubMed: 20133865]
148. Zhang XX, Eden HS, Chen X. J. Control. Release. 2011
149. Tkachenko AG, Xie H, Coleman D, Glomm W, Ryan J, Anderson MF, Franzen S, Feldheim DL. J. Am. Chem. Soc. 2003; 125:4700–4701. [PubMed: 12696875]
150. Smith GP, Petrenko VA. Chem. Rev. 1997; 97:391–410. [PubMed: 11848876]
151. Ruoslahti E, Bhatia SN, Sailor MJ. J. Cell Biol. 2010; 188:759–768. [PubMed: 20231381]
152. Ruoslahti E. Drug Discovery Today. 2002; 7:1138–1143. [PubMed: 12546857]
153. Iwasaki Y, Maie H, Akiyoshi K. Biomacromolecules. 2007; 8:3162–3168. [PubMed: 17883278]
154. Banquy X, Leclair G, Rabanel JM, Argaw A, Bouchard JF, Hildgen P, Giasson S. Bioconjugate Chem. 2008; 19:2030–2039.
155. Kim TH, Park IK, Nah JW, Choi YJ, Cho CS. Biomaterials. 2004; 25:3783–3792. [PubMed: 15020154]

156. Han HD, Mangala LS, Lee JW, Shahzad MM, Kim HS, Shen D, Nam EJ, Mora EM, Stone RL, Lu C, Lee SJ, Roh JW, Nick AM, Lopez-Berestein G, Sood AK. Clin. Cancer Res. 2010; 16:3910–3922. [PubMed: 20538762]
157. Aktas Y, Yemisci M, Andrieux K, Gursoy RN, Alonso MJ, Fernandez-Megia E, Novoa-Carballal R, Quinoa E, Riguera R, Sargon MF, Celik HH, Demir AS, Hincal AA, Dalkara T, Capan Y, Couvreur P. Bioconjugate Chem. 2005; 16:1503–1511.
158. Xu P, Tang H, Li S, Ren J, Van Kirk E, Murdoch WJ, Radosz M, Shen Y. Biomacromolecules. 2004; 5:1736–1744. [PubMed: 15360282]
159. Kim E, Kim D, Jung H, Lee J, Paul S, Selvapalam N, Yang Y, Lim N, Park CG, Kim K. Angew. Chem., Int. Ed. 2010; 49:4405–4408.
160. Bellocq NC, Pun SH, Jensen GS, Davis ME. Bioconjugate Chem. 2003; 14:1122–1132.
161. Beaudette TT, Cohen JA, Bachelder EM, Broaders KE, Cohen JL, Engleman EG, Frechet JM. J. Am. Chem. Soc. 2009; 131:10360–10361. [PubMed: 19591467]
162. Lee JB, Roh YH, Um SH, Funabashi H, Cheng W, Cha JJ, Kiatwuthinon P, Muller DA, Luo D. Nat. Nanotechnol. 2009; 4:430–436. [PubMed: 19581895]
163. MacKay JA, Chen M, McDaniel JR, Liu W, Simnick AJ, Chilkoti A. Nat. Mater. 2009; 8:993–999. [PubMed: 19898461]
164. Simnick AJ, Valencia CA, Liu R, Chilkoti A. ACS Nano. 2010; 4:2217–2227. [PubMed: 20334355]
165. Balthasar S, Michaelis K, Dinauer N, von Briesen H, Kreuter J, Langer K. Biomaterials. 2005; 26:2723–2732. [PubMed: 15585276]
166. Green JJ, Chiu E, Leshchiner ES, Shi J, Langer R, Anderson DG. Nano Lett. 2007; 7:874–879. [PubMed: 17362046]
167. Wang X, Li J, Wang Y, Cho KJ, Kim G, Gjyrezi A, Koenig L, Giannakakou P, Shin HJ, Tighiouart M, Nie S, Chen ZG, Shin DM. ACS Nano. 2009; 3:3165–3174. [PubMed: 19761191]
168. Choi KY, Chung H, Min KH, Yoon HY, Kim K, Park JH, Kwon IC, Jeong SY. Biomaterials. 2010; 31:106–114. [PubMed: 19783037]
169. Cho HJ, Yoon HY, Koo H, Ko SH, Shim JS, Lee JH, Kim K, Kwon IC, Kim DD. Biomaterials. 2011; 32:7181–7190. [PubMed: 21733572]
170. Nam HY, Kwon SM, Chung H, Lee SY, Kwon SH, Jeon H, Kim Y, Park JH, Kim J, Her S, Oh YK, Kwon IC, Kim K, Jeong SY. J. Controlled Release. 2009; 135:259–267.
171. Ryu JH, Jiwpanich S, Chacko R, Bickerton S, Thayumanavan S. J. Am. Chem. Soc. 2010; 132:8246–8247. [PubMed: 20504022]
172. Wang Y, Gao S, Ye WH, Yoon HS, Yang YY. Nat. Mater. 2006; 5:791–796. [PubMed: 16998471]
173. Shenoy D, Little S, Langer R, Amiji M. Pharm. Res. 2005; 22:2107–2114. [PubMed: 16254763]
174. Ko J, Park K, Kim YS, Kim MS, Han JK, Kim K, Park RW, Kim IS, Song HK, Lee DS, Kwon IC. J. Controlled Release. 2007; 123:109–115.
175. Cade D, Ramus E, Rinaudo M, Auzely-Velty R, Delair T, Hamaide T. Biomacromolecules. 2004; 5:922–927. [PubMed: 15132682]
176. Zhang L, Xue H, Cao Z, Keefe A, Wang J, Jiang S. Biomaterials. 2011; 32:4604–4608. [PubMed: 21453965]
177. Wang J, Tian S, Petros RA, Napier ME, Desimone JM. J. Am. Chem. Soc. 2010; 132:11306–11313. [PubMed: 20698697]
178. Lee H, Fonge H, Hoang B, Reilly RM, Allen C. Mol. Pharmaceutics. 2010; 7:1195–1208.
179. Xin H, Jiang X, Gu J, Sha X, Chen L, Law K, Chen Y, Wang X, Jiang Y, Fang X. Biomaterials. 2011; 32:4293–4305. [PubMed: 21427009]
180. Kaul G, Amiji M. Pharm. Res. 2005; 22:951–961. [PubMed: 15948039]
181. Nukolova NV, Oberoi HS, Cohen SM, Kabanov AV, Bronich TK. Biomaterials. 2011; 32:5417–5426. [PubMed: 21536326]
182. Nasongkla N, Bey E, Ren J, Ai H, Khemtong C, Guthi JS, Chin SF, Sherry AD, Boothman DA, Gao J. Nano Lett. 2006; 6:2427–2430. [PubMed: 17090068]

183. Harush-Frenkel O, Rozentur E, Benita S, Altschuler Y. Biomacromolecules. 2008; 9:435–443. [PubMed: 18189360]

184. Aryal S, Hu CM, Zhang L. ACS Nano. 2009; 4:251–258. [PubMed: 20039697]

185. McCarron PA, Marouf WM, Quinn DJ, Fay F, Burden RE, Olwill SA, Scott CJ. Bioconjugate Chem. 2008; 19:1561–1569.

186. Zhang N, Chittasupho C, Duangrat C, Siahaan TJ, Berkland C. Bioconjugate Chem. 2008; 19:145–152.

187. Tosi G, Fano RA, Bondioli L, Badiali L, Benassi R, Rivasi F, Ruozi B, Forni F, Vandelli MA. Nanomedicine. 2011; 6:423–436. [PubMed: 21542682]

188. Sengupta S, Eavarone D, Capila I, Zhao G, Watson N, Kiziltepe T, Sasisekharan R. Nature. 2005; 436:568–572. [PubMed: 16049491]

189. Townsend SA, Evrony GD, Gu FX, Schulz MP, Brown, Jr. RH, Langer R. Biomaterials. 2007; 28:5176–5184. [PubMed: 17854886]

190. Choi SW, Kim JH. J. Controlled Release. 2007; 122:24–30.

191. Cheng J, Teply BA, Sherifi I, Sung J, Luther G, Gu FX, Levy-Nissenbaum E, Radovic-Moreno AF, Langer R, Farokhzad OC. Biomaterials. 2007; 28:869–876. [PubMed: 17055572]

192. Dhar S, Gu FX, Langer R, Farokhzad OC, Lippard SJ. Proc. Natl. Acad. Sci. U. S. A. 2008; 105:17356–17361. [PubMed: 18978032]

193. Basu S, Harfouche R, Soni S, Chimote G, Mashelkar RA, Sengupta S. Proc. Natl. Acad. Sci. U. S. A. 2009; 106:7957–7961. [PubMed: 19383798]

194. Li J, Feng L, Fan L, Zha Y, Guo L, Zhang Q, Chen J, Pang Z, Wang Y, Jiang X, Yang VC, Wen L. Biomaterials. 2011; 32:4943–4950. [PubMed: 21470674]

195. Sanna V, Pintus G, Roggio AM, Punzoni S, Posadino AM, Arca A, Marceddu S, Bandiera P, Uzzau S, Sechi M. J. Med. Chem. 2011; 54:1321–1332. [PubMed: 21306166]

196. Gu F, Zhang L, Teply BA, Mann N, Wang A, Radovic- Moreno AF, Langer R, Farokhzad OC. Proc. Natl. Acad. Sci. U. S. A. 2008; 105:2586–2591. [PubMed: 18272481]

197. Poon Z, Chang D, Zhao X, Hammond PT. ACS Nano. 2011; 5:4284–4292. [PubMed: 21513353]

198. Rapoport N, Gao Z, Kennedy A. J. Natl. Cancer Inst. 2007; 99:1095–1106. [PubMed: 17623798]

199. Rothenfluh DA, Bermudez H, O'Neil CP, Hubbell JA. Nat. Mater. 2008; 7:248–254. [PubMed: 18246072]

200. Wei H, Zhang XZ, Zhou Y, Cheng SX, Zhuo RX. Biomaterials. 2006; 27:2028–2034. [PubMed: 16225918]

201. Schmidt V, Giacomelli C, Lecolley F, Lai-Kee-Him J, Brisson AR, Borsali R. J. Am. Chem. Soc. 2006; 128:9010–9011. [PubMed: 16834355]

202. Liang HF, Chen SC, Chen MC, Lee PW, Chen CT, Sung HW. Bioconjugate Chem. 2006; 17:291–299.

203. Yuan H, Luo K, Lai Y, Pu Y, He B, Wang G, Wu Y, Gu Z. Mol. Pharmaceutics. 2010; 7:953–962.

204. Lu J, Shi M, Shoichet MS. Bioconjugate Chem. 2009; 20:87–94.

205. Ding H, Inoue S, Ljubimov AV, Patil R, Portilla-Arias J, Hu J, Konda B, Wawrowsky KA, Fujita M, Karabalin N, Sasaki T, Black KL, Holler E, Ljubimova JY. Proc. Natl. Acad. Sci. U. S. A. 2010; 107:18143–18148. [PubMed: 20921419]

206. Inoue S, Ding H, Portilla-Arias J, Hu J, Konda B, Fujita M, Espinoza A, Suhane S, Riley M, Gates M, Patil R, Penichet ML, Ljubimov AV, Black KL, Holler E, Ljubimova JY. Cancer Res. 2011; 71:1454–1464. [PubMed: 21303974]

207. Cho CS, Kobayashi A, Takei R, Ishihara T, Maruyama A, Akaike T. Biomaterials. 2001; 22:45–51. [PubMed: 11085382]

208. Winer I, Wang S, Lee YE, Fan W, Gong Y, Burgos- Ojeda D, Spahlinger G, Kopelman R, Buckanovich RJ. Cancer Res. 2010; 70:8674–8683. [PubMed: 20959470]

209. Yao YC, Zhan XY, Zhang J, Zou XH, Wang ZH, Xiong YC, Chen J, Chen GQ. Biomaterials. 2008; 29:4823–4830. [PubMed: 18824258]

210. Na K, Bae YH. Pharm. Res. 2002; 19:681–688. [PubMed: 12069173]

211. Kickhoefer VA, Garcia Y, Mikyas Y, Johansson E, Zhou JC, Raval-Fernandes S, Minoofar P, Zink JI, Dunn B, Stewart PL, Rome LH. Proc. Natl. Acad. Sci. U. S. A. 2005; 102:4348–4352. [PubMed: 15753293]
212. Iyer AK, Greish K, Fang J, Murakami R, Maeda H. Biomaterials. 2007; 28:1871–1881. [PubMed: 17208294]
213. Matsumura Y, Gotoh M, Muro K, Yamada Y, Shirao K, Shimada Y, Okuwa M, Matsumoto S, Miyata Y, Ohkura H, Chin K, Baba S, Yamao T, Kannami A, Takamatsu Y, Ito K, Takahashi K. Ann. Oncol. 2004; 15:517–525. [PubMed: 14998859]
214. Hamaguchi T, Matsumura Y, Nakanishi Y, Muro K, Yamada Y, Shimada Y, Shirao K, Niki H, Hosokawa S, Tagawa T, Kakizoe T. Cancer Sci. 2004; 95:608–613. [PubMed: 15245599]
215. http://clinicaltrials.gov/ct2/show/NCT00470613
216. Sankhala KK, Mita AC, Adinin R, Wood L, Beeram M, Bullock S, Yamagata N, Matsuno K, Fujisawa T, Phan A. J. Clin. Oncol. 2009; 27:15S.
217. Daniels TR, Delgado T, Helguera G, Penichet ML. Clin. Immunol. 2006; 121:159–176. [PubMed: 16920030]
218. Gu F, Zhang L, Teply BA, Mann N, Wang A, Radovic- Moreno AF, Langer R, Farokhzad OC. Proc. Natl. Acad. Sci. U. S. A. 2008; 105:2586–2591. [PubMed: 18272481]
219. www.bindbio.com
220. Elsasser-Beile U, Buhler P, Wolf P. Curr. Drug Targets. 2009; 10:118–125. [PubMed: 19199907]
221. Slovin SF. Expert Opin. Ther. Targets. 2005; 9:561–570. [PubMed: 15948673]
222. Taxoteres®, B. prescribing information, sanofi-aventis. U.S. LLC, NJ: 2010.
223. Heidel JD, Liu JY, Yen Y, Zhou B, Heale BS, Rossi JJ, Bartlett DW, Davis ME. Clin. Cancer Res. 2007; 13:2207–2215. [PubMed: 17404105]
224. Bartlett DW, Davis ME. Bioconjugate Chem. 2007; 18:456–468.
225. Moreno AY, Janda KD. Pharmacol., Biochem. Behav. 2009; 92:199–205. [PubMed: 19350728]
226. Jung T, Breitenbach A, Kissel T. J. Controlled Release. 2000; 67:157–169.
227. Couvreur P, Kante B, Roland M, Guiot P, Bauduin P, Speiser P. J. Pharm. Pharmacol. 1979; 31:331–332. [PubMed: 37304]
228. Allemann E, Leroux JC, Gurny R, Doelker E. Pharm. Res. 1993; 10:1732–1737. [PubMed: 7905625]
229. Grabnar PA, Kristl J. J. Microencapsulation. 2011; 28:323–335. [PubMed: 21545323]
230. Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Int. J. Pharm. 1989; 55:R1–4.
231. Quintanar-Guerrero D, Allemann E, Fessi H, Doelker E. Drug Dev. Ind. Pharm. 1998; 24:1113–1128. [PubMed: 9876569]
232. Mora-Huertas CE, Fessi H, Elaissari A. Int. J. Pharm. 2010; 385:113–142. [PubMed: 19825408]
233. Farokhzad OC, Cheng JJ, Teply BA, Sherifi I, Jon S, Kantoff PW, Richie JP, Langer R. Proc. Natl. Acad. Sci. U. S. A. 2006; 103:6315–6320. [PubMed: 16606824]
234. Anton N, Benoit JP, Saulnier P. J. Controlled Release. 2008; 128:185–199.
235. Avgoustakis K. Curr. Drug Delivery. 2004; 1:321–333.
236. Karnik R, Gu F, Basto P, Cannizzaro C, Dean L, Kyei- Manu W, Langer R, Farokhzad OC. Nano Lett. 2008; 8:2906–2912. [PubMed: 18656990]
237. Rhee M, Valencia PM, Rodriguez MI, Langer R, Farokhzad OC, Karnik R. Adv. Mater. 2011; 23:H79–83. [PubMed: 21433105]
238. Whitesides GM. Nature. 2006; 442:368–373. [PubMed: 16871203]
239. DeMello AJ. Nature. 2006; 442:394–402. [PubMed: 16871207]
240. Marre S, Jensen KF. Chem. Soc. Rev. 2010; 39:1183–1202. [PubMed: 20179831]
241. Fraikin JL, Teesalu T, McKenney CM, Ruoslahti E, Cleland AN. Nat. Nanotechnol. 2011; 6:308–313. [PubMed: 21378975]
242. Gong J, Kim CJ. Lab Chip. 2008; 8:898–906. [PubMed: 18497909]
243. Wang H, Liu K, Chen KJ, Lu Y, Wang S, Lin WY, Guo F, Kamei K, Chen YC, Ohashi M, Wang M, Garcia MA, Zhao XZ, Shen CK, Tseng HR. ACS Nano. 2010; 4:6235–6243. [PubMed: 20925389]

244. Song H, Tice JD, Ismagilov RF. Angew. Chem., Int. Ed. 2003; 42:768–772.
245. Zhao CX, He LZ, Qiao SZ, Middelberg APJ. Chem. Eng. Sci. 2011; 66:1463–1479.
246. Dreis S, Rothweiler F, Michaelis M, Cinatl, Jr. J, Kreuter J, Langer K. Int. J. Pharm. 2007; 341:207–214. [PubMed: 17478065]
247. Kumar V, Prud'homme RK. J. Pharm. Sci. 2008; 97:4904–4914. [PubMed: 18300278]
248. Gindy ME, Panagiotopoulos AZ, Prud'homme RK. Langmuir. 2008; 24:83–90. [PubMed: 18044945]
249. Sanchis J, Canal F, Lucas R, Vicent MJ. Nanomedicine. 2010; 5:915–935. [PubMed: 20735226]
250. Greco F, Vicent MJ. Adv. Drug Delivery Rev. 2009; 61:1203–1213.
251. Duncan R. Adv. Drug Delivery Rev. 2009; 61:1131–1148.
252. Alexis F, Pridgen EM, Langer R, Farokhzad OC. Handb. Exp. Pharmacol. 2010:55–86. [PubMed: 20217526]
253. Tong R, Cheng J. Angew. Chem., Int. Ed. 2008; 47:4830–4834.
254. Kolishetti N, Dhar S, Valencia PM, Lin LQ, Karnik R, Lippard SJ, Langer R, Farokhzad OC. Proc. Natl. Acad. Sci. U. S. A. 2010; 107:17939–17944. [PubMed: 20921363]
255. Di Marco M, Shamsuddin S, Razak KA, Aziz AA, Devaux C, Borghi E, Levy L, Sadun C. Int. J. Nanomedicine. 2010; 5:37–49. [PubMed: 20161986]
256. Wang CH, Huang YF, Yeh CK. Langmuir. 2011; 27:6971–6976. [PubMed: 21553884]
257. Prescher JA, Bertozzi CR. Nat. Chem. Biol. 2005; 1:13–21. [PubMed: 16407987]
258. Canalle LA, van der Knaap M, Overhand M, van Hest JC. Macromol. Rapid Commun. 2011; 32:203–208. [PubMed: 21433141]
259. Guo J, Chen G, Ning X, Wolfert MA, Li X, Xu B, Boons GJ. Chem. Eur. J. 2010; 16:13360–13366. [PubMed: 21077066]
260. Debets MF, van der Doelen CW, Rutjes FP, van Delft FL. ChemBioChem. 2010; 11:1168–1184. [PubMed: 20455238]
261. Jewett JC, Bertozzi CR. Chem. Soc. Rev. 2010; 39:1272–1279. [PubMed: 20349533]
262. Devaraj NK, Upadhyay R, Haun JB, Hilderbrand SA, Weissleder R. Angew. Chem., Int. Ed. 2009; 48:7013–7016.
263. Tassa C, Shaw SY, Weissleder R. Acc. Chem. Res. 2011; 44:842–852. [PubMed: 21661727]
264. Haun JB, Devaraj NK, Marinelli BS, Lee H, Weissleder R. ACS Nano. 2011; 5:3204–3213. [PubMed: 21351804]
265. Algar WR, Prasuhn DE, Stewart MH, Jennings TL, Blanco-Canosa JB, Dawson PE, Medintz IL. Bioconjugate Chem. 2011; 22:825–858.
266. Valencia PM, Hanewich-Hollatz MH, Gao WW, Karim F, Langer R, Karnik R, Farokhzad OC. Biomaterials. 2011; 32:6226–6233. [PubMed: 21658757]
267. Nobs L, Buchegger F, Gurny R, Allemann E. Bioconjugate Chem. 2006; 17:139–145.
268. Nobs L, Buchegger F, Gurny R, Allemann E. Eur. J. Pharm. Biopharm. 2004; 58:483–490. [PubMed: 15451522]
269. Brennan FR, Shaw L, Wing MG, Robinson C. Mol. Biotechnol. 2004; 27:59–74. [PubMed: 15122047]
270. Weinberg WC, Frazier-Jessen MR, Wu WJ, Weir A, Hartsough M, Keegan P, Fuchs C. Cancer Metastasis Rev. 2005; 24:569–584. [PubMed: 16408162]
271. Orlova A, Tolmachev V, Pehrson R, Lindborg M, Tran T, Sandstrom M, Nilsson FY, Wennborg A, Abrahmsen L, Feldwisch J. Cancer Res. 2007; 67:2178–2186. [PubMed: 17332348]
272. Willuda J, Honegger A, Waibel R, Schubiger PA, Stahel R, Zangemeister-Wittke U, Pluckthun A. Cancer Res. 1999; 59:5758–5767. [PubMed: 10582696]
273. Cortez-Retamozo V, Backmann N, Senter PD, Wernery U, De Baetselier P, Muyldermans S, Revets H. Cancer Res. 2004; 64:2853–2857. [PubMed: 15087403]
274. Chou LY, Ming K, Chan WC. Chem. Soc. Rev. 2011; 40:233–245. [PubMed: 20886124]
275. Choi CH, Alabi CA, Webster P, Davis ME. Proc. Natl. Acad. Sci. U. S. A. 2010; 107:1235–1240. [PubMed: 20080552]

276. Thomas TP, Shukla R, Kotlyar A, Liang B, Ye JY, Norris TB, Baker, Jr. JR. Biomacromolecules. 2008; 9:603–609. [PubMed: 18193839]

277. Cui B, Wu C, Chen L, Ramirez A, Bearer EL, Li WP, Mobley WC, Chu S. Proc. Natl. Acad. Sci. U. S. A. 2007; 104:13666–13671. [PubMed: 17698956]

278. Norberto SE, Kakar SS. Exp. Mol. Pathol. 2009

279. Chang DK, Lin CT, Wu CH, Wu HC. PLoS One. 2009; 4:e4171. [PubMed: 19137069]

280. Negussie AH, Miller JL, Reddy G, Drake SK, Wood BJ, Dreher MR. J. Controlled Release. 2010; 143:265–273.

281. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Greenwald DR, Ruoslahti E. Science. 2010; 328:1031–1035. [PubMed: 20378772]

282. Farokhzad OC, Jon S, Khademhosseini A, Tran TN, Lavan DA, Langer R. Cancer Res. 2004; 64:7668–7672. [PubMed: 15520166]

283. Huang YF, Shangguan D, Liu H, Phillips JA, Zhang X, Chen Y, Tan W. ChemBioChem. 2009; 10:862–868. [PubMed: 19253922]

284. Huang CC, Huang YF, Cao Z, Tan W, Chang HT. Anal. Chem. 2005; 77:5735–5741. [PubMed: 16131089]

285. Herr JK, Smith JE, Medley CD, Shangguan D, Tan W. Anal. Chem. 2006; 78:2918–2924. [PubMed: 16642976]

286. Toma A, Otsuji E, Kuriu Y, Okamoto K, Ichikawa D, Hagiwara A, Ito H, Nishimura T, Yamagishi H. Br. J. Cancer. 2005; 93:131–136. [PubMed: 15970924]

287. Gosk S, Vermehren C, Storm G, Moos T. J. Cereb. Blood Flow Metab. 2004; 24:1193–1204. [PubMed: 15545912]

288. Wagner S, Rothweiler F, Anhorn MG, Sauer D, Riemann I, Weiss EC, Katsen-Globa A, Michaelis M, Cinatl, Jr. J, Schwartz D, Kreuter J, von Briesen H, Langer K. Biomaterials. 2010; 31:2388–2398. [PubMed: 20031203]

289. Torchilin VP, Lukyanov AN, Gao Z, Papahadjopoulos-Sternberg B. Proc. Natl. Acad. Sci. U. S. A. 2003; 100:6039–6044. [PubMed: 12716967]

290. Sawant RM, Cohen MB, Torchilin VP, Rokhlin OW. J. Drug Targeting. 2008; 16:601–604.

291. Laginha KM, Moase EH, Yu N, Huang A, Allen TM. J. Drug Targeting. 2008; 16:605–610.

292. Voinea M, Manduteanu I, Dragomir E, Capraru M, Simionescu M. Pharm. Res. 2005; 22:1906–1917. [PubMed: 16088429]

293. Loomis K, Smith B, Feng Y, Garg H, Yavlovich A, Campbell-Massa R, Dimitrov DS, Blumenthal R, Xiao X, Puri A. Exp. Mol. Pathol. 2010; 88:238–249. [PubMed: 20122924]

294. Beuttler J, Rothdiener M, Muller D, Frejd FY, Kontermann RE. Bioconjugate Chem. 2009; 20:1201–1208.

295. Silverman J, Liu Q, Bakker A, To W, Duguay A, Alba BM, Smith R, Rivas A, Li P, Le H, Whitehorn E, Moore KW, Swimmer C, Perlroth V, Vogt M, Kolkman J, Stemmer WP. Nat. Biotechnol. 2005; 23:1556–1561. [PubMed: 16299519]

296. Burg M, Ravey EP, Gonzales M, Amburn E, Faix PH, Baird A, Larocca D. DNA Cell Biol. 2004; 23:457–462. [PubMed: 15294095]

297. Liu B, Conrad F, Cooperberg MR, Kirpotin DB, Marks JD. Cancer Res. 2004; 64:704–710. [PubMed: 14744788]

298. Wan XM, Chen YP, Xu WR, Yang WJ, Wen LP. Peptides. 2009; 30:343–350. [PubMed: 19007831]

299. van Rooy I, Cakir-Tascioglu S, Couraud PO, Romero IA, Weksler B, Storm G, Hennink WE, Schiffelers RM, Mastrobattista E. Pharm. Res. 2010; 27:673–682. [PubMed: 20162339]

300. Stern ST, McNeil SE. Toxicol. Sci. 2008; 101:4–21. [PubMed: 17602205]

301. Gu FX, Karnik R, Wang AZ, Alexis F, Levy-Nissenbaum E, Hong S, Langer RS, Farokhzad OC. Nano Today. 2007; 2:14–21.

302. Brissette R, Prendergast JK, Goldstein NI. Curr. Opin. Drug Discovery Dev. 2006; 9:363–369.

303. Krag DN, Shukla GS, Shen GP, Pero S, Ashikaga T, Fuller S, Weaver DL, Burdette-Radoux S, Thomas C. Cancer Res. 2006; 66:7724–7733. [PubMed: 16885375]

304. Desgrosellier JS, Cheresh DA. Nat. Rev. Cancer. 2010; 10:9–22. [PubMed: 20029421]
305. Randolph JT, Waid P, Nichols C, Sauer D, Haviv F, Diaz G, Bammert G, Besecke LM, Segreti JA, Mohning KM, Bush EN, Wegner CD, Greer J. J. Med. Chem. 2004; 47:1085–1097. [PubMed: 14971889]
306. Cortijo J, Sanz MJ, Iranzo A, Montesinos JL, Nabah YN, Alfon J, Gomez LA, Merlos M, Morcillo EJ. Br. J. Pharmacol. 2006; 147:661–670. [PubMed: 16432509]
307. Chan JM, Rhee JW, Drum CL, Bronson RT, Golomb G, Langer R, Farokhzad OC. Proc. Natl. Acad. Sci. U. S. A. 2011; 108:19347–19352. [PubMed: 22087004]
308. Arap W, Kolonin MG, Trepel M, Lahdenranta J, Cardo- Vila M, Giordano RJ, Mintz PJ, Ardelt PU, Yao VJ, Vidal CI, Chen L, Flamm A, Valtanen H, Weavind LM, Hicks ME, Pollock RE, Botz GH, Bucana CD, Koivunen E, Cahill D, Troncoso P, Baggerly KA, Pentz RD, Do KA, Logothetis CJ, Pasqualini R. Nat. Med. 2002; 8:121–127. [PubMed: 11821895]
309. Pasqualini R, Koivunen E, Kain R, Lahdenranta J, Sakamoto M, Stryhn A, Ashmun RA, Shapiro LH, Arap W, Ruoslahti E. Cancer Res. 2000; 60:722–727. [PubMed: 10676659]
310. Jain RK, Stylianopoulos T. Nat. Rev. Clin. Oncol. 2010; 7:653–664. [PubMed: 20838415]
311. Heitz F, Morris MC, Divita G. Br. J. Pharmacol. 2009; 157:195–206. [PubMed: 19309362]
312. Ruan G, Agrawal A, Marcus AI, Nie S. J. Am. Chem. Soc. 2007; 129:14759–14766. [PubMed: 17983227]
313. Gump JM, Dowdy SF. Trends Mol. Med. 2007; 13:443–448. [PubMed: 17913584]
314. Sugahara KN, Teesalu T, Karmali PP, Kotamraju VR, Agemy L, Girard OM, Hanahan D, Mattrey RF, Ruoslahti E. Cancer Cell. 2009; 16:510–520. [PubMed: 19962669]
315. Laakkonen P, Porkka K, Hoffman JA, Ruoslahti E. Nat. Med. 2002; 8:751–755. [PubMed: 12053175]
316. Porkka K, Laakkonen P, Hoffman JA, Bernasconi M, Ruoslahti E. Proc. Natl. Acad. Sci. U. S. A. 2002; 99:7444–7449. [PubMed: 12032302]
317. Wilson DS, Szostak JW. Annu. Rev. Biochem. 1999; 68:611–647. [PubMed: 10872462]
318. Tuerk C, Gold L. Science. 1990; 249:505–510. [PubMed: 2200121]
319. Ellington AD, Szostak JW. Nature. 1990; 346:818–822. [PubMed: 1697402]
320. Keefe AD, Pai S, Ellington A. Nat. Rev. Drug Discovery. 2010; 9:537–550.
321. Mayer G. Angew. Chem., Int. Ed. 2009; 48:2672–2689.
322. Jayasena SD. Clin. Chem. 1999; 45:1628–1650. [PubMed: 10471678]
323. Cohen C, Forzan M, Sproat B, Pantophlet R, McGowan I, Burton D, James W. Virology. 2008; 381:46–54. [PubMed: 18799178]
324. Bagalkot V, Farokhzad OC, Langer R, Jon S. Angew. Chem., Int. Ed. 2006; 45:8149–8152.
325. Guo J, Gao X, Su L, Xia H, Gu G, Pang Z, Jiang X, Yao L, Chen J, Chen H. Biomaterials. 2011; 32:8010–8020. [PubMed: 21788069]
326. Wu Z, Tang LJ, Zhang XB, Jiang JH, Tan W. ACS Nano. 2011; 5:7696–7699. [PubMed: 22023403]
327. Drabovich AP, Berezovski MV, Musheev MU, Krylov SN. Anal. Chem. 2009; 81:490–494. [PubMed: 19055427]
328. Talekar M, Kendall J, Denny W, Garg S. Anti-Cancer Drugs. 2011; 22:949–962. [PubMed: 21970851]
329. Xia W, Low PS. J. Med. Chem. 2010; 53:6811–6824. [PubMed: 20666486]
330. Leamon CP, Reddy JA. Adv. Drug Delivery Rev. 2004; 56:1127–1141.
331. Reddy JA, Dorton R, Westrick E, Dawson A, Smith T, Xu LC, Vetzel M, Kleindl P, Vlahov IR, Leamon CP. Cancer Res. 2007; 67:4434–4442. [PubMed: 17483358]
332. Liang C, Yang Y, Ling Y, Huang Y, Li T, Li X. Bioorg. Med. Chem. 2011; 19:4057–4066. [PubMed: 21641806]
333. Hilgenbrink AR, Low PS. J. Pharm. Sci. 2005; 94:2135–2146. [PubMed: 16136558]
334. Kamaly N, Kalber T, Thanou M, Bell JD, Miller AD. Bioconjugate Chem. 2009; 20:648–655.
335. Ohguchi Y, Kawano K, Hattori Y, Maitani Y. J. Drug Targeting. 2008; 16:660–667.

336. Chandran SS, Banerjee SR, Mease RC, Pomper MG, Denmeade SR. Cancer Biol. Ther. 2008; 7:974–982. [PubMed: 18698158]
337. Chen Y, Dhara S, Banerjee SR, Byun Y, Pullambhatla M, Mease andM. G. Pomper RC. Biochem. Biophys. Res. Commun. 2009; 390:624–629. [PubMed: 19818734]
338. Farokhzad OC, Jon SY, Khadelmhosseini A, Tran TNT, LaVan DA, Langer R. Cancer Res. 2004; 64:7668–7672. [PubMed: 15520166]
339. Wijagkanalan W, Kawakami S, Hashida M. Front. Biosci. 2011; 17:2970–2987. [PubMed: 21622215]
340. Hashida M, Nishikawa M, Yamashita F, Takakura Y. Adv. Drug Delivery Rev. 2001; 52:187–196.
341. Li X, Zhou H, Yang L, Du G, Pai-Panandiker AS, Huang X, Yan B. Biomaterials. 2011; 32:2540–2545. [PubMed: 21232787]
342. Bergen JM, von Recum HA, Goodman TT, Massey AP, Pun SH. Macromol. Biosci. 2006; 6:506–516. [PubMed: 16921538]
343. Sawyers CL. Nature. 2008; 452:548–552. [PubMed: 18385728]
344. Guo KT, Ziemer G, Paul A, Wendel HP. Int. J. Mol. Sci. 2008; 9:668–678. [PubMed: 19325777]
345. de Kruif J, Terstappen L, Boel E, Logtenberg T. Proc. Natl. Acad. Sci. U. S. A. 1995; 92:3938–3942. [PubMed: 7537380]
346. Fang X, Tan W. Acc. Chem. Res. 2009; 43:48–57. [PubMed: 19751057]
347. Shangguan D, Cao ZC, Li Y, Tan W. Clin. Chem. 2007; 53:1153–1155. [PubMed: 17463173]
348. Pasqualini R, Ruoslahti E. Nature. 1996; 380:364–366. [PubMed: 8598934]
349. Arap W, Haedicke W, Bernasconi M, Kain R, Rajotte D, Krajewski S, Ellerby HM, Bredesen DE, Pasqualini R, Ruoslahti E. Proc. Natl. Acad. Sci. U. S. A. 2002; 99:1527–1531. [PubMed: 11830668]
350. Mi J, Liu Y, Rabbani ZN, Yang Z, Urban JH, Sullenger BA, Clary BM. Nat. Chem. Biol. 2010; 6:22–24. [PubMed: 19946274]
351. Xiao Z, Levy-Nissenbaum E, Alexis F, Luptak A, Teply BA, Chan JM, Shi J, Digga E, Cheng J, Langer R, Farokhzad OC. ACS Nano. 2012; 6:696–704. [PubMed: 22214176]
352. http://cen.acs.org/articles/90/web/2012/01/Aptamer-Guided-Nanoparticles-Slip-Cells.html
353. Nel AE, Madler L, Velegol D, Xia T, Hoek EM, Somasundaran P, Klaessig F, Castranova V, Thompson M. Nat. Mater. 2009; 8:543–557. [PubMed: 19525947]
354. Wang S, Dormidontova EE. Biomacromolecules. 2010; 11:1785–1795. [PubMed: 20536119]
355. Tassa C, Duffner JL, Lewis TA, Weissleder R, Schreiber SL, Koehler AN, Shaw SY. Bioconjugate Chem. 2010; 21:14–19.
356. Fakhari A, Baoum A, Siahaan TJ, Le KB, Berkland C. J. Pharm. Sci. 2011; 100:1045–1056. [PubMed: 20922813]
357. Adiseshaiah PP, Hall JB, McNeil SE. Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol. 2010; 2:99–112. [PubMed: 20049834]
358. Conner SD, Schmid SL. Nature. 2003; 422:37–44. [PubMed: 12621426]
359. Zhao F, Zhao Y, Liu Y, Chang X, Chen C. Small. 2011; 7:1322–1337. [PubMed: 21520409]
360. Wang J, Byrne JD, Napier ME, Desimone JM. Small. 2011; 7:1919–1931. [PubMed: 21695781]
361. Verma A, Stellacci F. Small. 2010; 6:12–21. [PubMed: 19844908]
362. Gratton SE, Ropp PA, Pohlhaus PD, Luft JC, Madden VJ, Napier ME, DeSimone JM. Proc. Natl. Acad. Sci. U. S. A. 2008; 105:11613–11618. [PubMed: 18697944]
363. He C, Hu Y, Yin L, Tang C, Yin C. Biomaterials. 2010; 31:3657–3666. [PubMed: 20138662]
364. Jiang W, Kim BY, Rutka JT, Chan WC. Nat. Nanotechnol. 2008; 3:145–150. [PubMed: 18654486]
365. Vonarbourg A, Passirani C, Saulnier P, Simard P, Leroux JC, Benoit JP. J. Biomed. Mater. Res. A. 2006; 78:620–628. [PubMed: 16779767]
366. Petros RA, DeSimone JM. Nat. Rev. Drug Discovery. 2010; 9:615–627.
367. Moghimi SM, Hunter AC, Murray JC. Pharmacol. Rev. 2001; 53:283–318. [PubMed: 11356986]

368. Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Itty Ipe B, Bawendi MG, Frangioni JV. Nat. Biotechnol. 2007; 25:1165–1170. [PubMed: 17891134]
369. Torchilin VP. AAPS J. 2007; 9:E128–E147. [PubMed: 17614355]
370. Wong C, Stylianopoulos T, Cui J, Martin J, Chauhan VP, Jiang W, Popovic Z, Jain RK, Bawendi MG, Fukumura D. Proc. Natl. Acad. Sci. U. S. A. 2011; 108:2426–2431. [PubMed: 21245339]
371. Schadlich A, Caysa H, Mueller T, Tenambergen F, Rose C, Gopferich A, Kuntsche J, Mader K. ACS Nano. 2011; 5:8710–8720. [PubMed: 21970766]
372. Rhee M, Valencia PM, Rodriguez MI, Langer R, Farokhzad OC, Karnik R. Adv. Mater. 2011; 23:H79–H83. [PubMed: 21433105]
373. Geng Y, Dalhaimer P, Cai S, Tsai R, Tewari M, Minko T, Discher DE. Nat. Nanotechnol. 2007; 2:249–255. [PubMed: 18654271]
374. Decuzzi P, Godin B, Tanaka T, Lee SY, Chiappini C, Liu X, Ferrari M. J. Controlled Release. 2010; 141:320–327.
375. Dausend J, Musyanovych A, Dass M, Walther P, Schrezenmeier H, Landfester K, Mailander V. Macromol. Biosci. 2008; 8:1135–1143. [PubMed: 18698581]
376. Salvador-Morales C, Zhang L, Langer R, Farokhzad OC. Biomaterials. 2009; 30:2231–2240. [PubMed: 19167749]
377. Valencia PM, Basto PA, Zhang L, Rhee M, Langer R, Farokhzad OC, Karnik R. ACS Nano. 2010; 4:1671–1679. [PubMed: 20166699]
378. Vonarbourg A, Passirani C, Saulnier P, Benoit JP. Biomaterials. 2006; 27:4356–4373. [PubMed: 16650890]
379. Hoek EM, Agarwal GK. J. Colloid Interface Sci. 2006; 298:50–58. [PubMed: 16469325]
380. Shastri VP, Lipski AM, Jaquiery C, Choi H, Eberli D, Stevens M, Martin I, Chen IW. Adv. Mater. 2007; 19:553.
381. Merkel TJ, Jones SW, Herlihy KP, Kersey FR, Shields AR, Napier M, Luft JC, Wu H, Zamboni WC, Wang AZ, Bear JE, DeSimone JM. Proc. Natl. Acad. Sci. U. S. A. 2011; 108:586–591. [PubMed: 21220299]
382. Anderson NL, Polanski M, Pieper R, Gatlin T, Tirumalai RS, Conrads TP, Veenstra TD, Adkins JN, Pounds JG, Fagan R, Lobley A. Mol. Cell. Proteomics. 2004; 3:311–326. [PubMed: 14718574]
383. Ashley CE, Carnes EC, Phillips GK, Padilla D, Durfee PN, Brown PA, Hanna TN, Liu J, Phillips B, Carter MB, Carroll NJ, Jiang X, Dunphy DR, Willman CL, Petsev DN, Evans DG, Parikh AN, Chackerian B, Wharton W, Peabody DS, Brinker CJ. Nat. Mater. 2011; 10:389–397. [PubMed: 21499315]
384. Aggarwal P, Hall JB, McLeland CB, Dobrovolskaia MA, McNeil SE. Adv. Drug Delivery Rev. 2009; 61:428–437.
385. Moghimi SM, Szebeni J. Prog. Lipid Res. 2003; 42:463–478. [PubMed: 14559067]
386. Scherphof GL, Velinova M, Kamps J, Donga J. Liposome research days: Towards new products for human health, Shizuoka, Japan. 1996
387. Perrault SD, Walkey C, Jennings T, Fischer HC, Chan WC. Nano Lett. 2009; 9:1909–1915. [PubMed: 19344179]
388. Owens DE 3rd, Peppas NA. Int. J. Pharm. 2006; 307:93–102. [PubMed: 16303268]
389. Makarucha AJ, Todorova N, Yarovsky I. Eur. Biophys. J. 2011; 40:103–115. [PubMed: 21153635]
390. Kluza E, van der Schaft DW, Hautvast PA, Mulder WJ, Mayo KH, Griffioen AW, Strijkers GJ, Nicolay K. Nano Lett. 2010; 10:52–58. [PubMed: 19968235]
391. Patil YB, Toti US, Khdair A, Ma L, Panyam J. Biomaterials. 2009; 30:859–866. [PubMed: 19019427]
392. Siegwart DJ, Whitehead KA, Nuhn L, Sahay G, Cheng H, Jiang S, Ma M, Lytton-Jean A, Vegas A, Fenton P, Levins CG, Love KT, Lee H, Cortez C, Collins SP, Li YF, Jang J, Querbes W, Zurenko C, Novobrantseva T, Langer R, Anderson DG. Proc. Natl. Acad. Sci. U. S. A. 2011; 108:12996–13001. [PubMed: 21784981]

393. Weissleder R, Kelly K, Sun EY, Shtatland T, Josephson L. Nat. Biotechnol. 2005; 23:1418–1423. [PubMed: 16244656]
394. Hrkach J, Von Hoff D, Ali M, Andrianova E, Auer J, Campbell T, de Witt D, Figa M, Figueiredo M, Horhota A, Low S, McDonell K, Peeke E, Retnarajan B, Sabnis A, Schnipper E, Song J, Song YH, Summa J, Tompsett D, Troiano G, van Geen T, Wright J, LoRusso P, Kantoff PW, Bander NH, Sweeney CJ, Farokhzad OC, Langer R, Zale S. Preclinical Development and Clinical Translation of a PSMA-Targeted Docetaxel Nanoparticle with a Differentiated Pharmacological Profile. Sci. Transl. Med. 2012 (in press).
395. Zhang L, Radovic-Moreno AF, Alexis F, Gu FX, Basto PA, Bagalkot V, Jon S, Langer RS, Farokhzad OC. ChemMedChem. 2007; 2:1268–1271. [PubMed: 17600796]
396. Milane L, Duan Z, Amiji M. PLoS One. 2011; 6:e24075. [PubMed: 21931642]
397. Hu CM, Zhang L. Biochem. Pharmacol. 2012 Jan 18.
398. Hu C, Aryal S, Zhang L. Ther. Delivery. 2010; 1:323–334.
399. Chan JM, Zhang L, Yuet KP, Liao G, Rhee JW, Langer R, Farokhzad OC. Biomaterials. 2009; 30:1627–1634. [PubMed: 19111339]
400. Shi J, Xiao Z, Votruba AR, Vilos C, Farokhzad OC. Angew. Chem., Int. Ed. 2011; 50:7027–7031.
401. Torchilin VP. Annu. Rev. Biomed. Eng. 2006; 8:343–375. [PubMed: 16834560]
402. Li S-D, Huang L. Mol. Pharmaceutics. 2008; 5:496–504.
403. Kaufmann AM, Krise JP. J. Pharm. Sci. 2007; 96:729–746. [PubMed: 17117426]
404. Panyam J, Labhasetwar V. Adv. Drug Delivery Rev. 2003; 55:329–347.
405. Weissig V. Crit. Rev. Ther. Drug Carrier Syst. 2003; 20:1–62. [PubMed: 12911263]
406. Weissig V. Expert Opin. Drug Delivery. 2005; 2:89–102.
407. Weissig V, Boddapati SV, Jabr L, D'Souza GG. Nanomedicine. 2007; 2:275–285. [PubMed: 17716174]
408. Minton AP. J. Cell Sci. 2006; 119:2863–2869. [PubMed: 16825427]
409. Ellis RJ, Minton AP. Nature. 2003; 425:27–28. [PubMed: 12955122]
410. Kim D, Lee ES, Oh KT, Gao ZG, Bae YH. Small. 2008; 4:2043–2050. [PubMed: 18949788]
411. Cho K, Wang X, Nie S, Chen ZG, Shin DM. Clin. Cancer Res. 2008; 14:1310–1316. [PubMed: 18316549]
412. Bareford LM, Swaan PW. Adv. Drug Delivery Rev. 2007; 59:748–758.
413. Tate BA, Mathews PM. Sci. Aging Knowl. Environ. 2006:re2.
414. Castino R, Demoz M, Isidoro C. J. Mol. Recognit. 2003; 16:337–348. [PubMed: 14523947]
415. Lim CS. Adv. Drug Delivery Rev. 2007; 59:697.
416. Seibel P, Trappe J, Villani G, Klopstock T, Papa S, Reichmann H. Nucleic Acids Res. 1995; 23:10–17. [PubMed: 7870573]
417. Weissig V, Torchilin VP. Adv. Drug Delivery Rev. 2001; 49:127–149.
418. Qian ZM, Li H, Sun H, Ho K. Pharmacol. Rev. 2002; 54:561–587. [PubMed: 12429868]
419. Eavarone DA, Yu X, Bellamkonda RV. J. Biomed. Mater. Res. 2000; 51:10–14. [PubMed: 10813739]
420. Dufes J-M, Muller C, Couet J-C, Olivier W, Uchegbu IF, Schaetzlein AG. Pharm. Res. 2004; 21:101–107. [PubMed: 14984263]
421. Muro S, Schuchman EH, Muzykantov VR. Mol. Ther. 2006; 13:135–141. [PubMed: 16153895]
422. Lee M, Choi JS, Choi MJ, Pak YK, Rhee BD, Ko KS. J. Drug Targeting. 2007; 15:115–122.
423. Murakami M, Cabral H, Matsumoto Y, Wu S, Kano MR, Yamori T, Nishiyama N, Kataoka K. Sci. Transl. Med. 2011; 3:64ra62.
424. Preiss MR, Bothun GD. Expert Opin. Drug Delivery. 2011; 8:1025–1040.
425. Delcea M, Mohwald H, Skirtach AG. Adv. Drug Delivery Rev. 2011; 63:730–747.
426. Gao WW, Chan JM, Farokhzad OC. Mol. Pharmaceutics. 2010; 7:1913–1920.
427. Hribar KC, Lee MH, Lee D, Burdick JA. ACS Nano. 2011; 5:2948–2956. [PubMed: 21384864]

428. Peng K, Tomatsu I, van den Broek B, Cui C, Korobko AV, van Noort J, Meijer AH, Spaink HP, Kros A. Soft Matter. 2011; 7:4881–4887.
429. la Rica RD, Aili D, Stevens MM. Adv. Drug Delivery Rev. 2011
430. Thornton PD, Heise A. J. Am. Chem. Soc. 2010; 132:2024–2028. [PubMed: 20099895]
431. Zhu LJ, Tu CL, Zhu BS, Su Y, Pang Y, Yan DY, Wu JL, Zhu XY. Polym. Chem. 2011; 2:1761–1768.
432. Esser-Kahn AP, Odom SA, Sottos NR, White SR, Moore JS. Macromolecules. 2011; 44:5539–5553.
433. Jin Y, Song L, Su Y, Zhu LJ, Pang Y, Qiu F, Tong GS, Yan DY, Zhu BS, Zhu XY. Biomacromolecules. 2011; 12:3460–3468. [PubMed: 21863891]
434. O'Neill BE, Rapoport N. Ther. Delivery. 2011; 2:1165–1187.
435. Mamasheva E, O'Donnell C, Bandekar A, Sofou S. Mol. Pharmaceutics. 2011; 8:2224–2232.
436. Chen D, Liu W, Shen Y, Mu H, Zhang Y, Liang R, Wang A, Sun K, Fu F. Int. J. Nanomed. 2011; 6:2053–2061.
437. Yin H, Lee ES, Kim D, Lee KH, Oh KT, Bae YH. J. Controlled Release. 2008; 126:130–138.
438. Lee ES, Gao Z, Kim D, Park K, Kwon IC, Bae YH. J. Controlled Release. 2008; 129:228–236.
439. Schwarze SR, Ho A, Vocero-Akbani A, Dowdy SF. Science. 1999; 285:1569–1572. [PubMed: 10477521]
440. Chung JE, Yokoyama M, Yamato M, Aoyagi T, Sakurai Y, Okano T. J. Controlled Release. 1999; 62:115–127.
441. Zhang L, Chan JM, Gu FX, Rhee JW, Wang AZ, Radovic-Moreno AF, Alexis F, Langer R, Farokhzad OC. ACS Nano. 2008; 2:1696–1702. [PubMed: 19206374]
442. Katsogiannou M, Peng L, Catapano CV, Rocchi P. Curr. Cancer Drug Targets. 2011; 11:954–965. [PubMed: 21861840]
443. Alexis F, Pridgen E, Molnar LK, Farokhzad OC. Mol. Pharmaceutics. 2008; 5:505–515.
444. Feynman RP. Caltec Engineering and Science. 1960; 23:22–36.

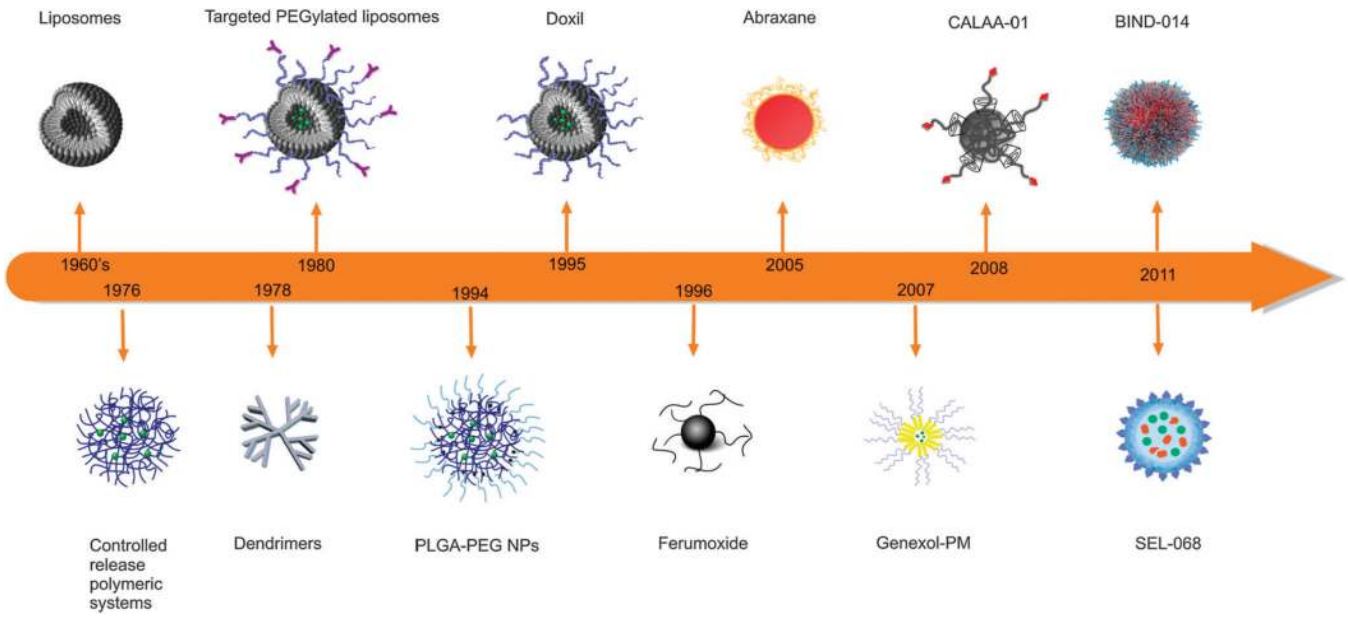

Fig. 1.

Time line of clinical stage nanomedicine firsts. Liposomes,[9] controlled release polymeric systems for macromolecules,[10] dendrimers,[11] targeted-PEGylated liposomes,[12] first FDA approved liposome (DOXIL),[13] long circulating poly(lactic-*co*-glycolic acid)-polyethyleneglycol (PLGA-PEG) NPs,[14] iron oxide MRI contrast agent NP (Ferumoxide),[15] protein based drug delivery system (Abraxane; nab technologyt),[16] polymeric micelle NP (Genexol-PM),[17] targeted cyclodextrin-polymer hybrid NP (CALAA-01),[18] targeted polymeric NP (BIND-014; Accurint™ Technology),[19] fully integrated polymeric nanoparticle vaccines (SEL-068, *t* SVPt™ Technology).[20]

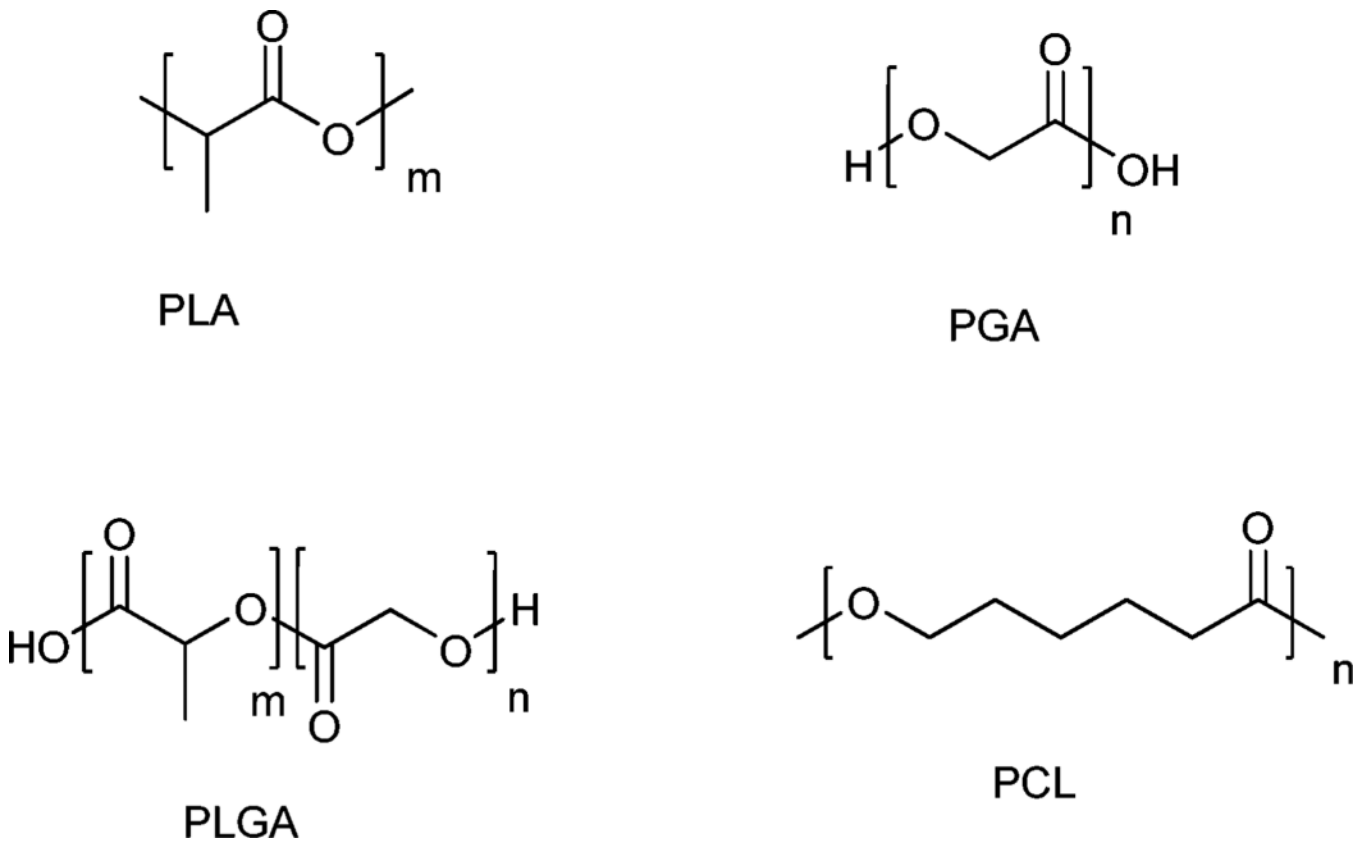

Fig. 2.
Common biodegradable polymers utilized in controlled-release drug delivery applications. Poly(lactic acid) (PLA), poly(glutamic acid) (PGA), poly(D,L-lactic-*co*-glycolide) (PLGA), poly(caprolactone) (PCL).

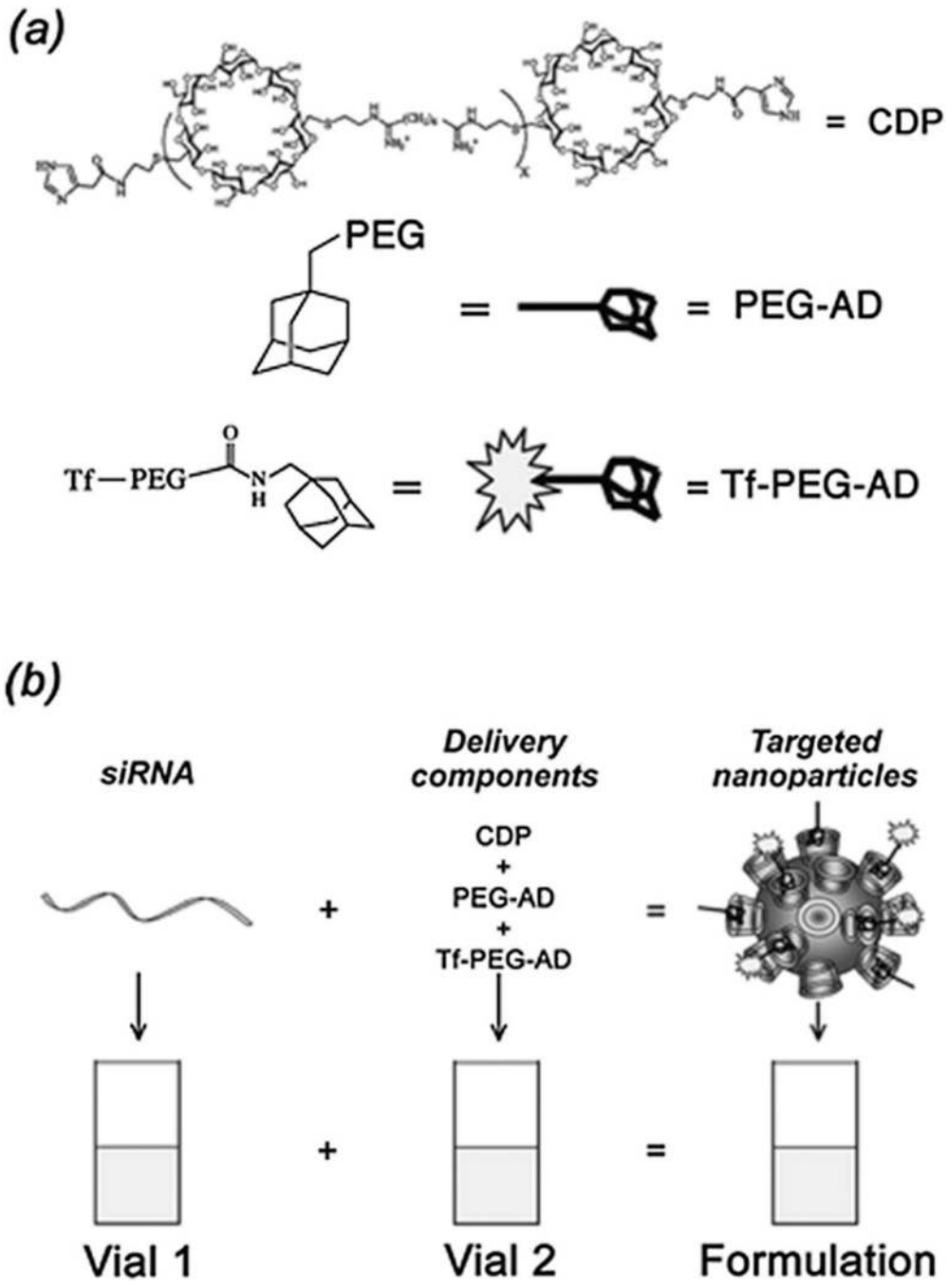

Fig. 3.

Components of CALAA-01 (Calando Pharmaceuticals-01) – a targeted NP for siRNA delivery: (a) CDP: water-soluble, linear cyclodextrin-containing polymer, AD: adamantane (AD)-PEG conjugate (PEG MW of 5000) (AD-PEG), and Tf-PEG-AD: an adamantane conjugate of PEG (PEGMWof 5000) conjugated with human transferrin (Tf) ligand. (b) CALAA-01 is formulated *via* a single self-assembly process of four individual components. Figure taken from Davis, M *et al.*[18]

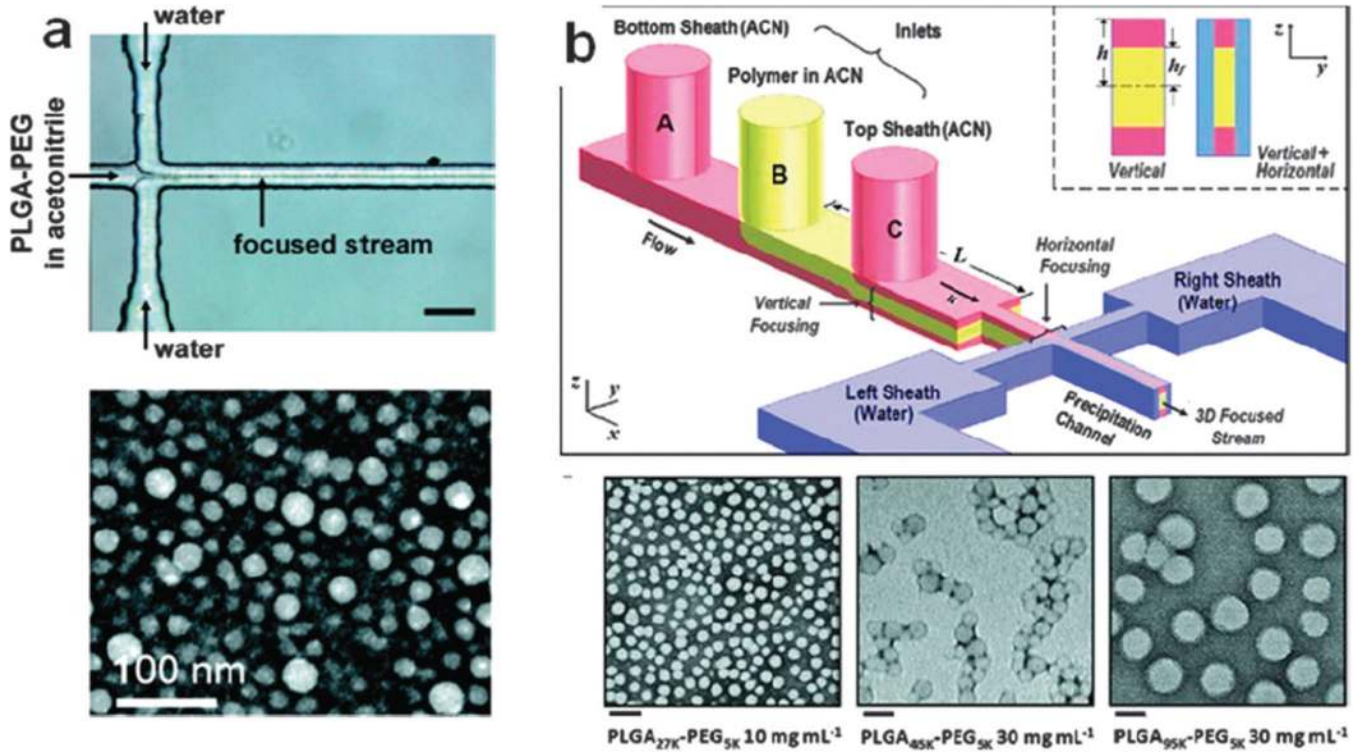

Fig. 4.

(a) Microfluidic synthesis of polymeric nanoparticles prepared under rapid mixing conditions in 2D flow focusing. (b) 3D flow focusing. Figure adapted from Karnik *et al.* and Rhee *et al.*[236, 237]

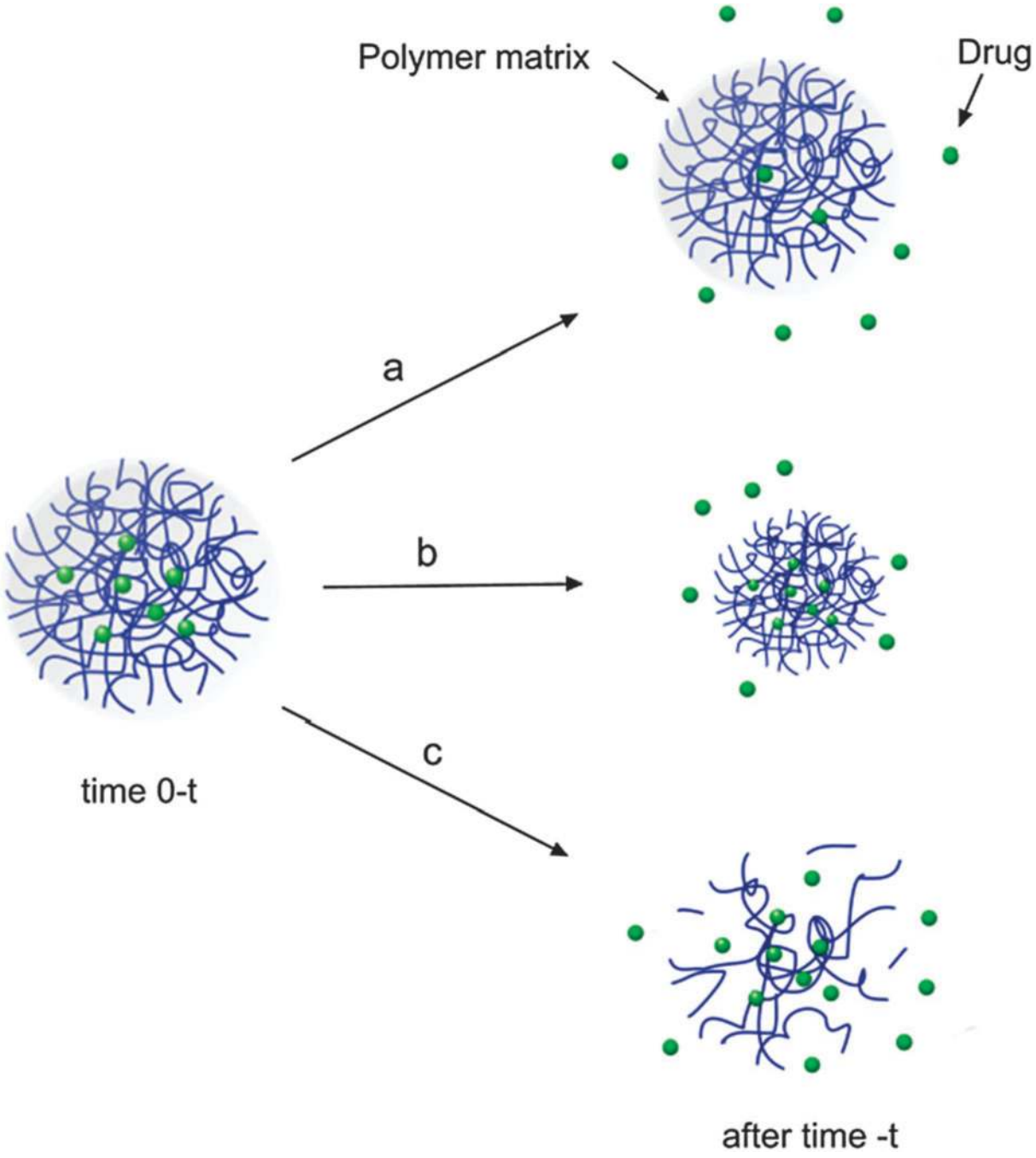

Fig. 5.
Drug release mechanisms from polymeric NPs: (a) diffusion from polymer matrix with time varying diffusivity, (b) surface erosion/degradation of polymer matrix, and (c) biodegradation of polymer matrix due to hydrolytic degradation leading to drug release.

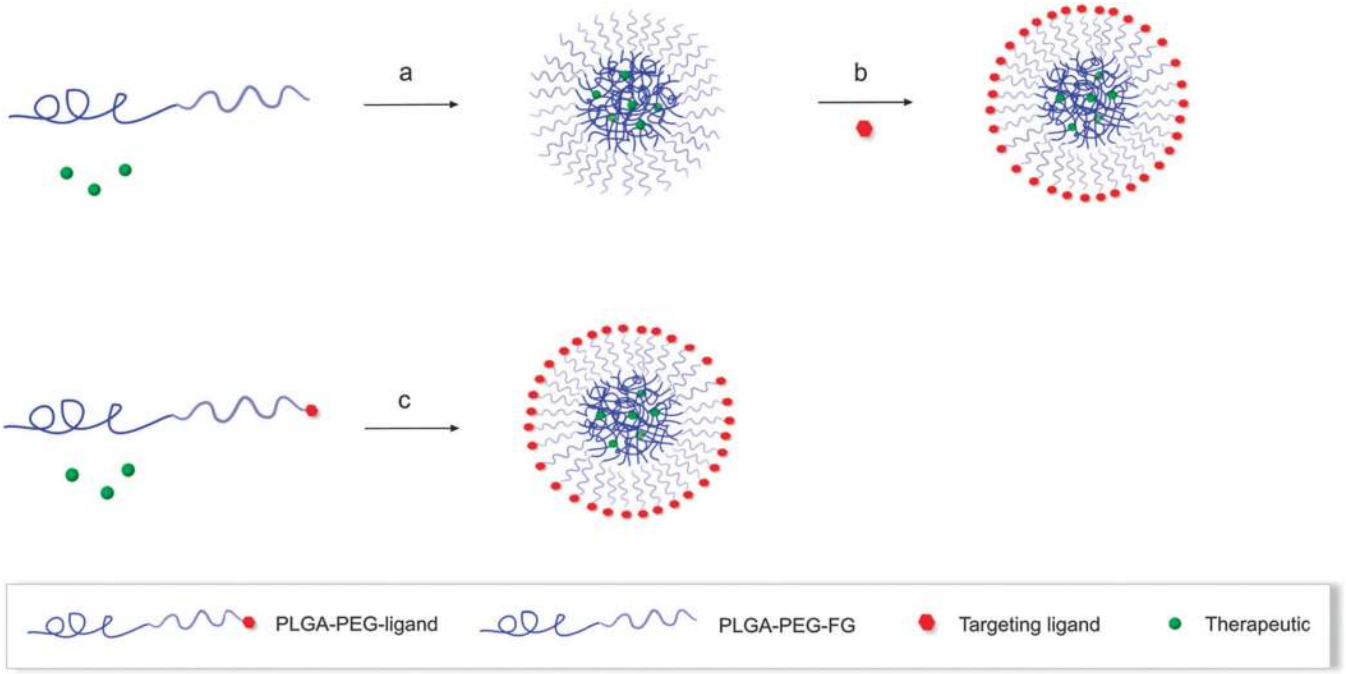

Fig. 6.

Self-assembly of triblock PLGA-*b*-PEG copolymers in aqueous solution: (a) Polymeric NP formation *via* nanoprecipitation, FG = functional group (b) Conjugation of targeting ligand to the surface of pre-formed polymeric NPs (c) Pre-functionalized diblock polymer with hydrophilic targeting ligand.

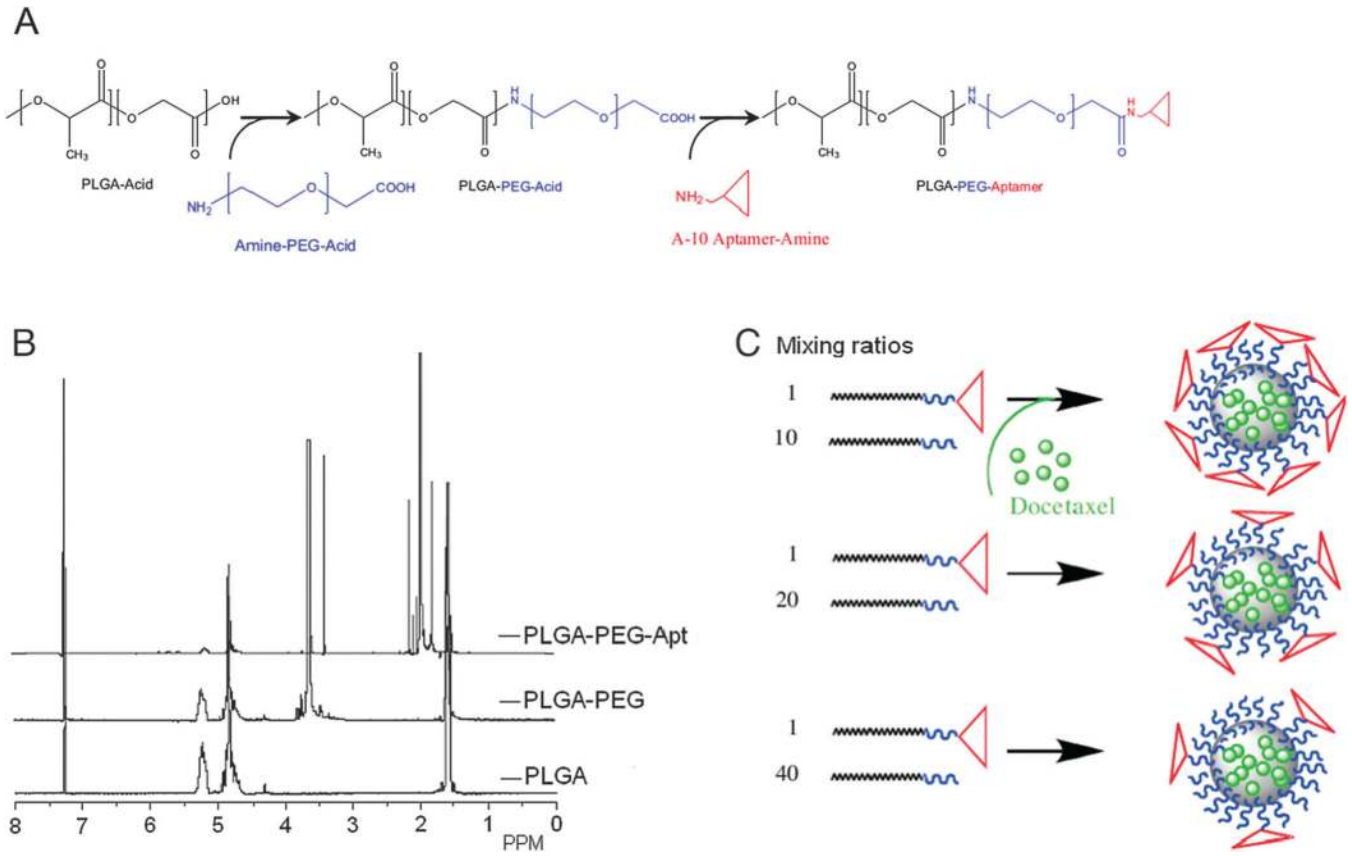

Fig. 7.

Self-assembling targeted polymeric NPs. A-B: Synthesis and characterization of PLGA-PEG-Apt triblock polymers. C: Nanoprecipitation leading to the self-assembly of PLGA-PEG-Apt NPs. Aptamer surface density is precisely controlled using distinct ratios of PLGA-PEG-Apt and PLGA- PEG. Figure taken from Gu *et al.*[218]

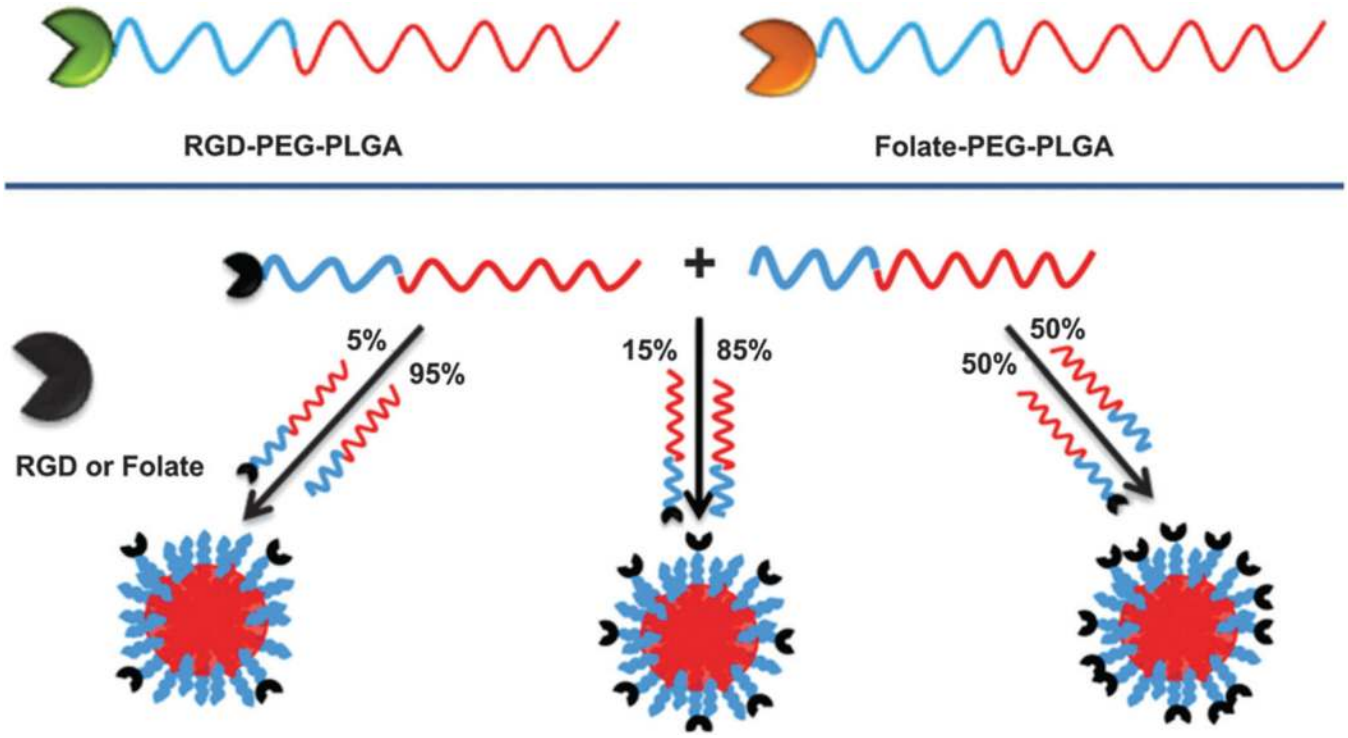

Fig. 8.
PLGA-PEG-RGD and PLGA-PEG-folate triblock polymers used to prepare targeted NPs with different surface ligand densities. Figure taken from Valencia *et al.*[266]

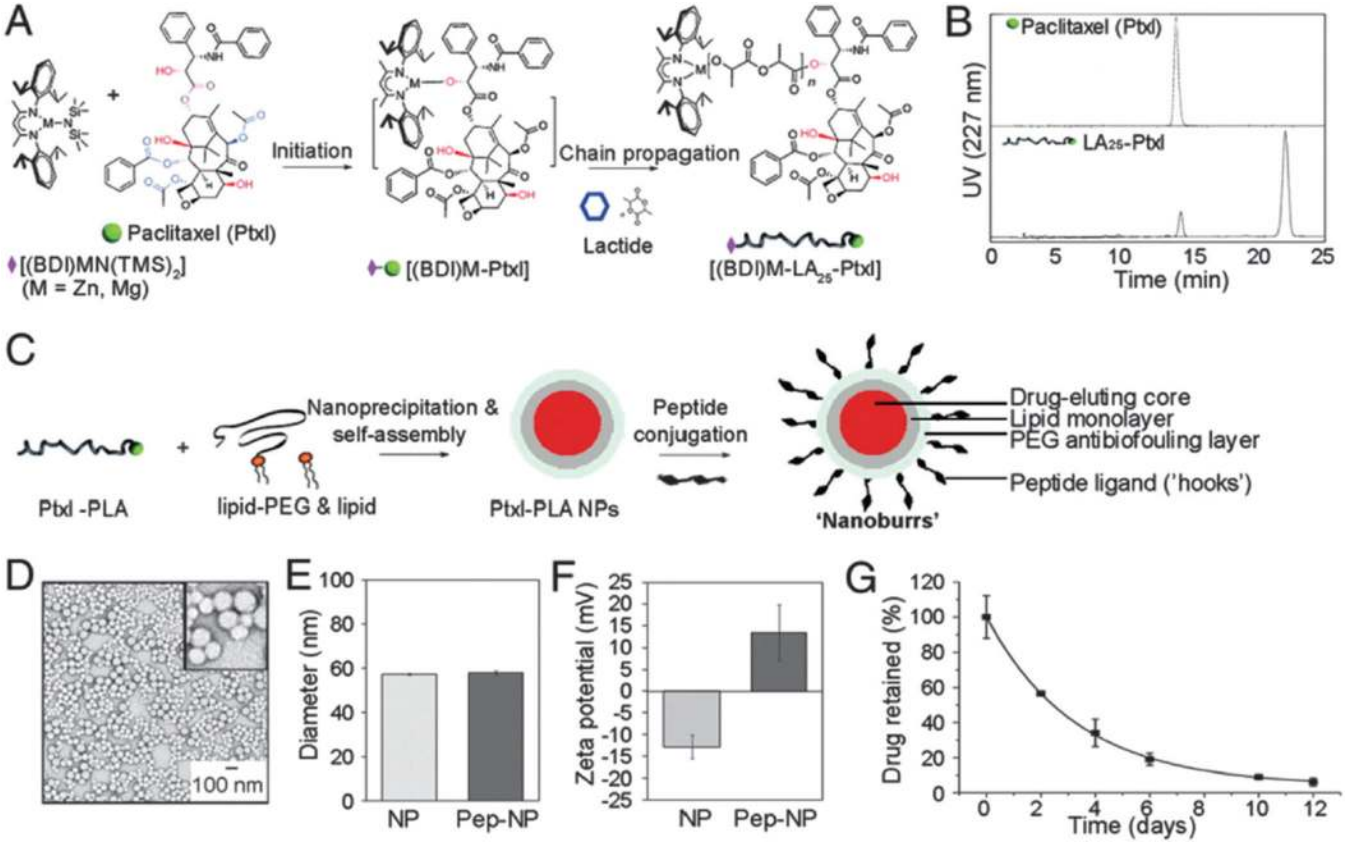

Fig. 9.

Polymer-lipid hybrid 'Nanoburr' particles. A: Polymer-drug conjugate synthesis (Ptxl-PLA), B: HPLC characterization of Ptxl and Ptxl-PLA polymer, C: Nanoburr synthesis *via* polymer lipid, and lipid-PEG self-assembly, D: TEM image of Nanoburrs (stained with 3% uranyl acetate), E: Dynamic light scattering measurements (DLS) pre and post peptide conjugation, F: Zeta potential measurements pre and post peptide onjugation, and G: *in vitro* drug release profile of Ptxl from Nanoburr NPs. Figure taken from Chan *et al.*[147]

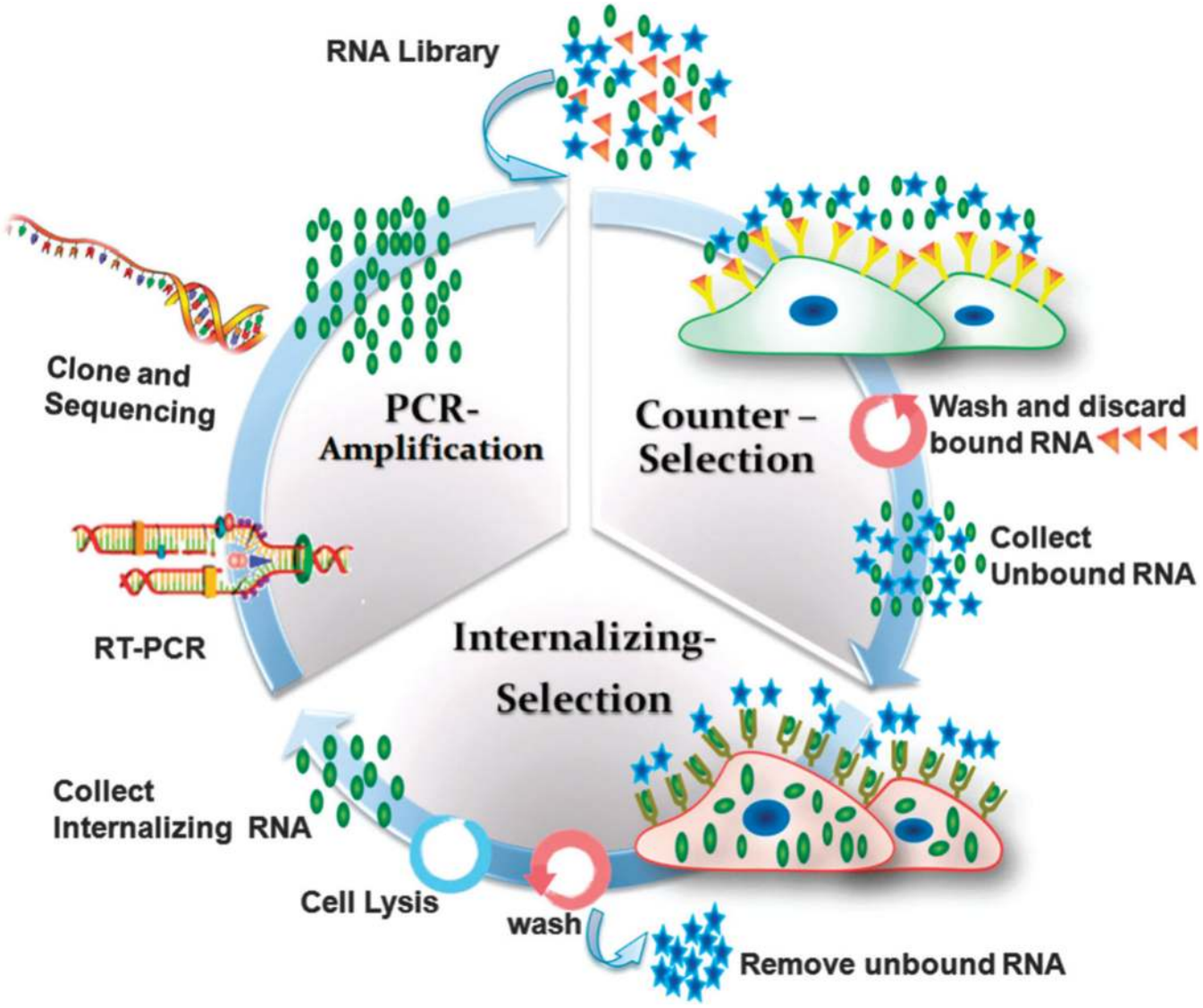

Fig. 10.
Schematic protocol of cell-uptake selection for evolving cancer cell-specific internalizing Apts. Figure taken from Xiao *et al.*[351]

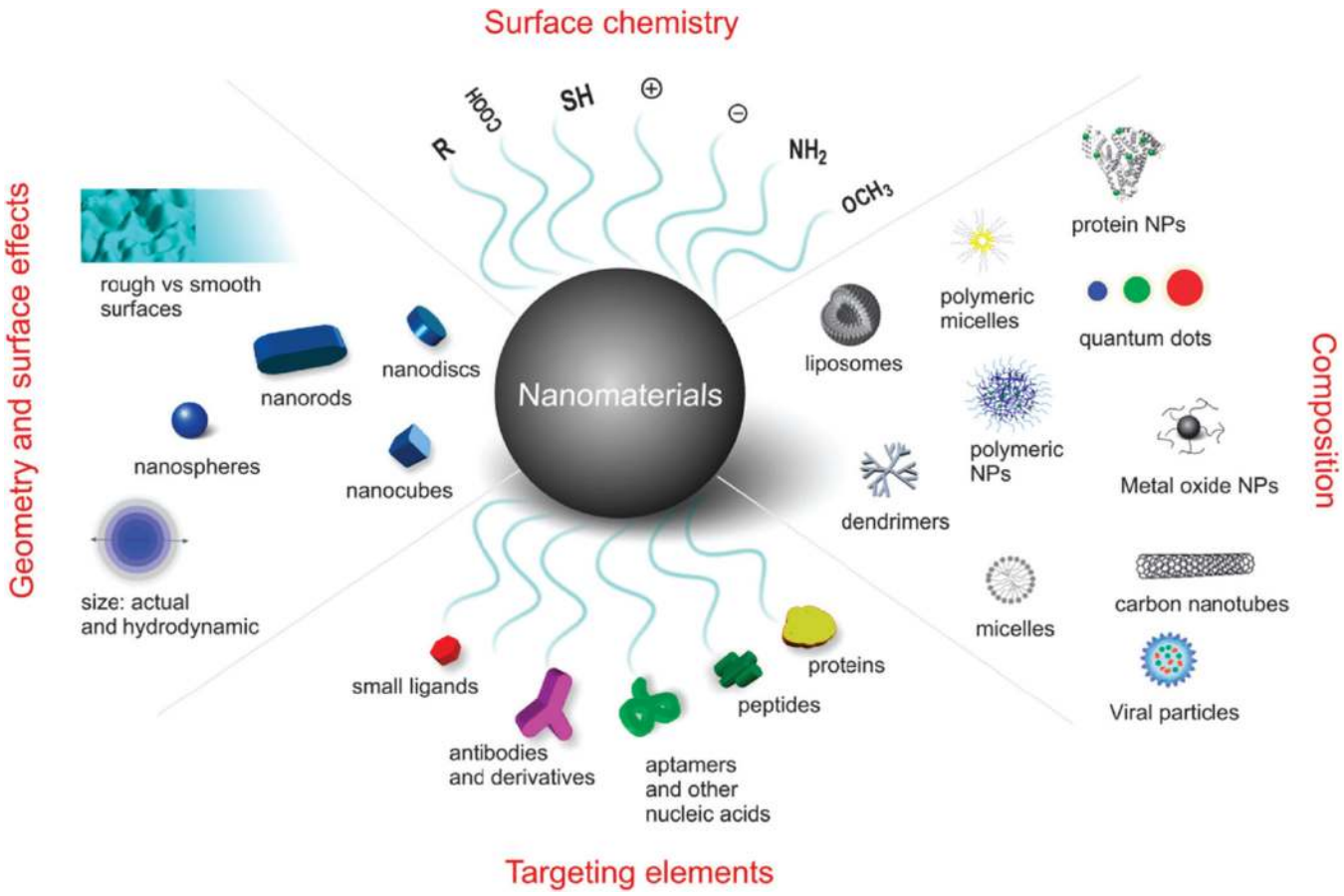

Fig. 11.

NPs and their biophysicochemical characteristics which affect their performance both *in vitro* and *in vivo*.

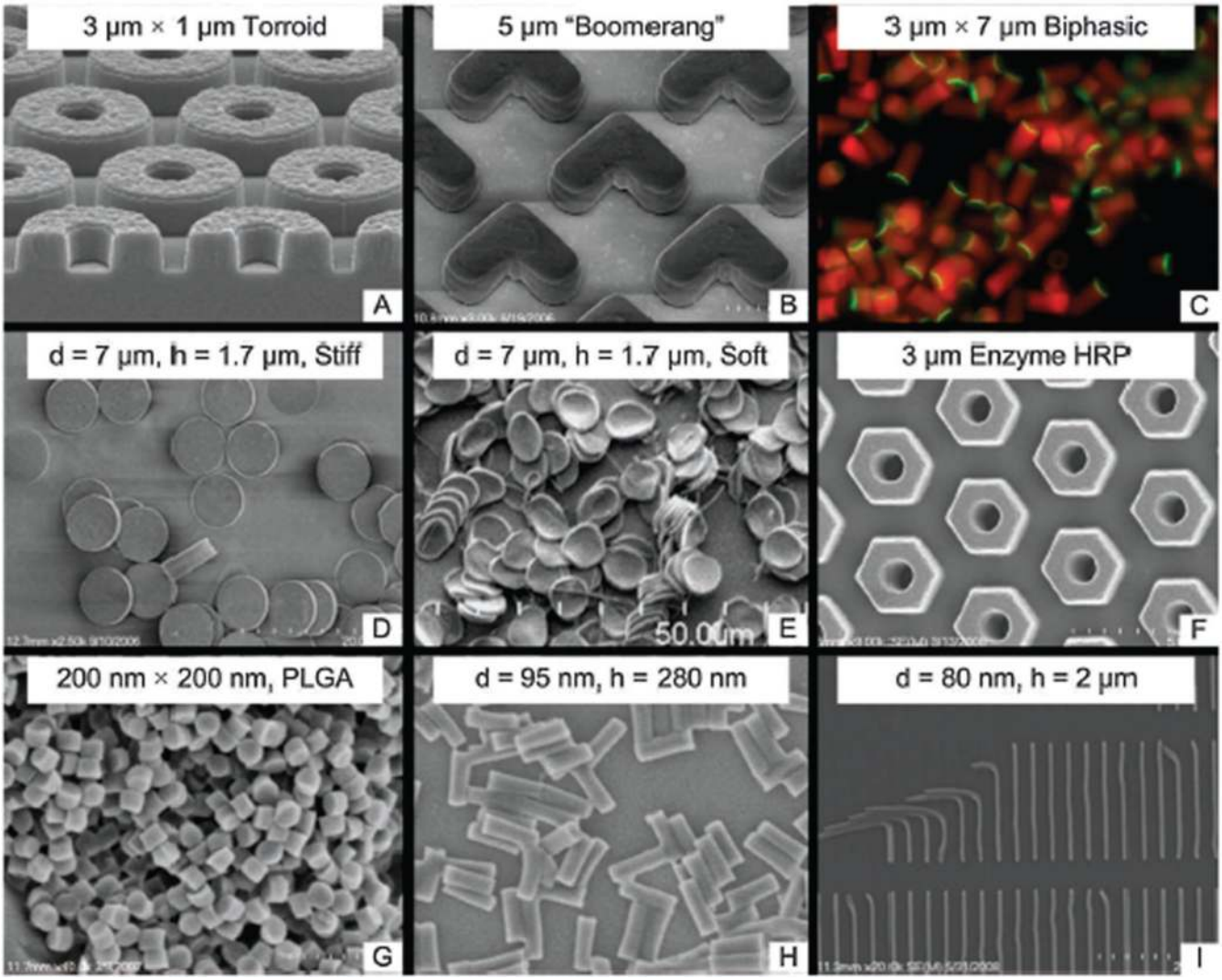

Fig. 12.
Development of an array of nano and microparticles with variable shapes and aspect ratios using the PRINT technique by Desimone *et al.* Figure taken from Wang *et al.*[360]

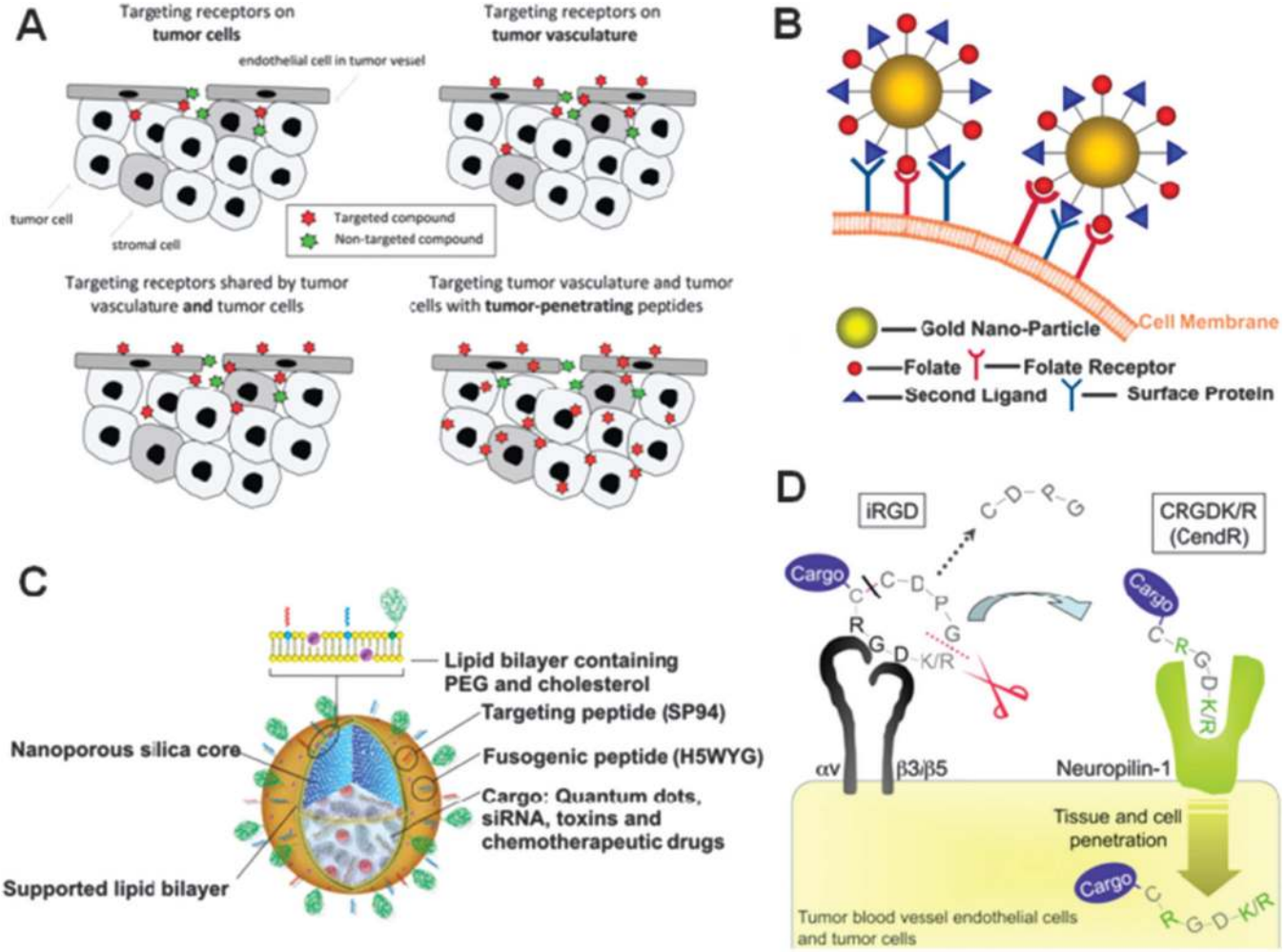

Fig. 13.

Different strategies for multi-ligand targeting of NPs: A: Various modes of targeting using single or multi-ligands (Figure taken from Ruoslahti *et al.*[260]), B: Dual-targeting where one NP has two different ligands that target receptors on the same cell (Figure adapted from Li *et al.*[341]), C: example of cellular and sub-cellular targeting (Figure taken from Ashley *et al.*[383]), and D: dual targeting of one peptide to two different receptors on the same cell (Figure adapted from K. Sugahara *et al.*[257]).

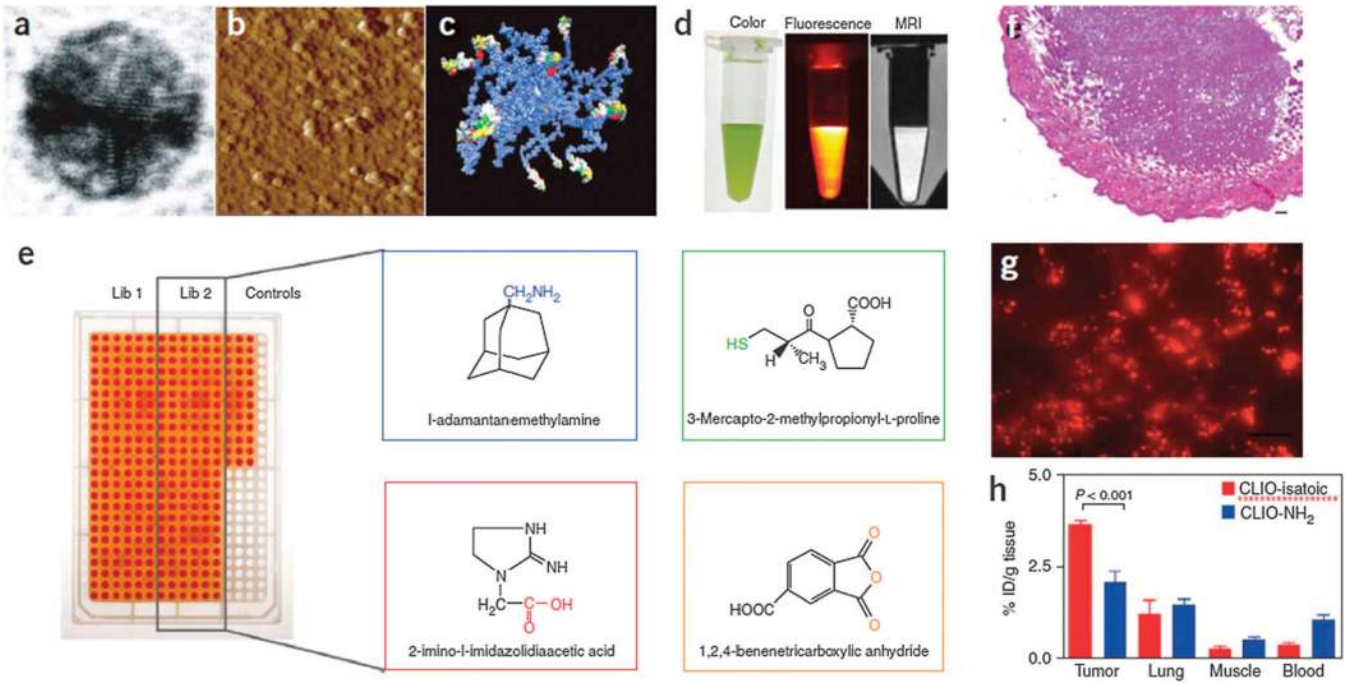

Fig. 14.

Screening targeted NPs from a derived NP library: (a–b) Laser light scattering and atomic force microscopy of NPs. (c) Model of the crosslinked dextran coating NPs modified with small molecules. (d) Water solubility, as well as fluorescent and magnetic properties of NPs. (e) Different classes of small molecules with amino, sulfhydryl, carboxyl or anhydride functionalities anchored onto the NPs. (f) Hemotoxylin eosin–stained sections of the tumours targeting with NPs. (g) Tumour cross-sections observed using the Cy5.5 fluorescence channel indicate marked fluorescence of one identified NP; CLIO-isatoic within tumour cells. (h) Biodistribution study with ^{111}In-labelled NPs confirmed tumoural targeting of CLIO-isatoic NPs. Figure adapted from Weissleder *et al.*[393]

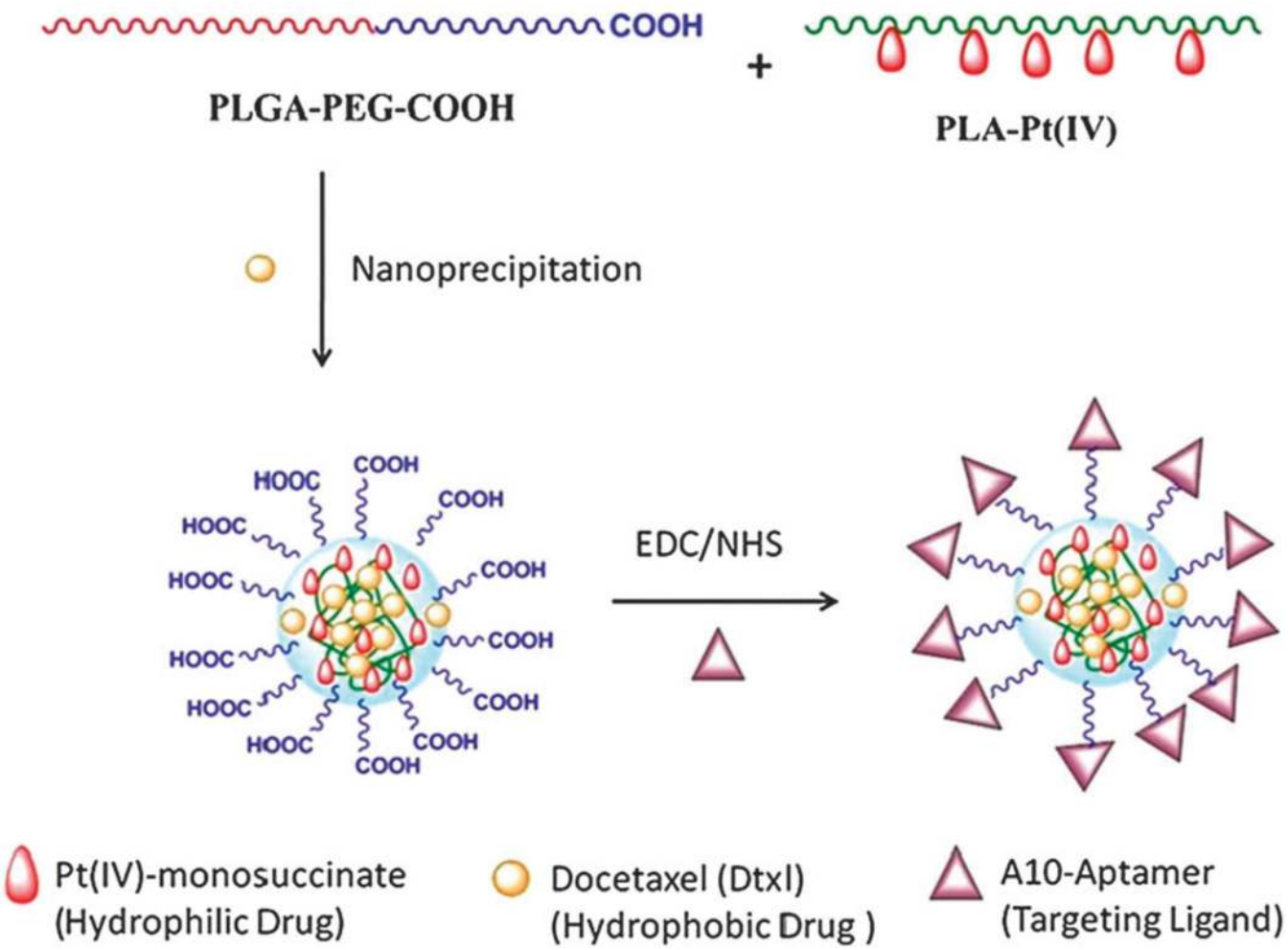

Fig. 15.
Strategy for co-encapsulating hydrophobic Dtxl and more hydrophilic Pt(IV)-monosuccinate prodrug on a single nanoparticle. Figure taken from Kolishetti *et al.*[46]

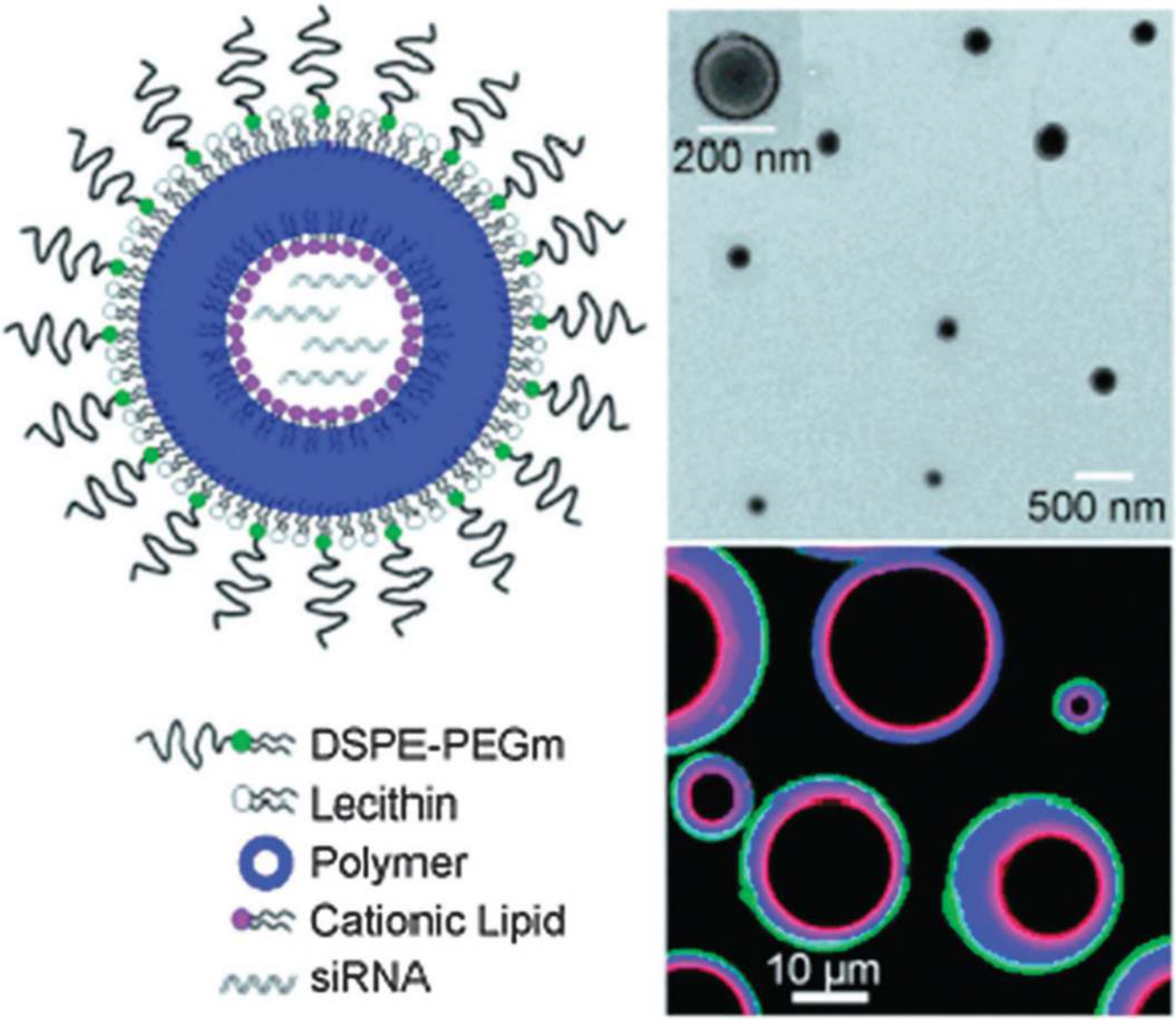

Fig. 16.
Biodegradable and biocompatible polymers and lipids forming hybrid core/shell nanoparticles for siRNA delivery. The unique lipid–polymer–lipid nanostructure is demonstrated by TEM (top right) and fluorescence microscopy (bottom right) with microsized particles. Figure taken from Shi *et al.*[400]

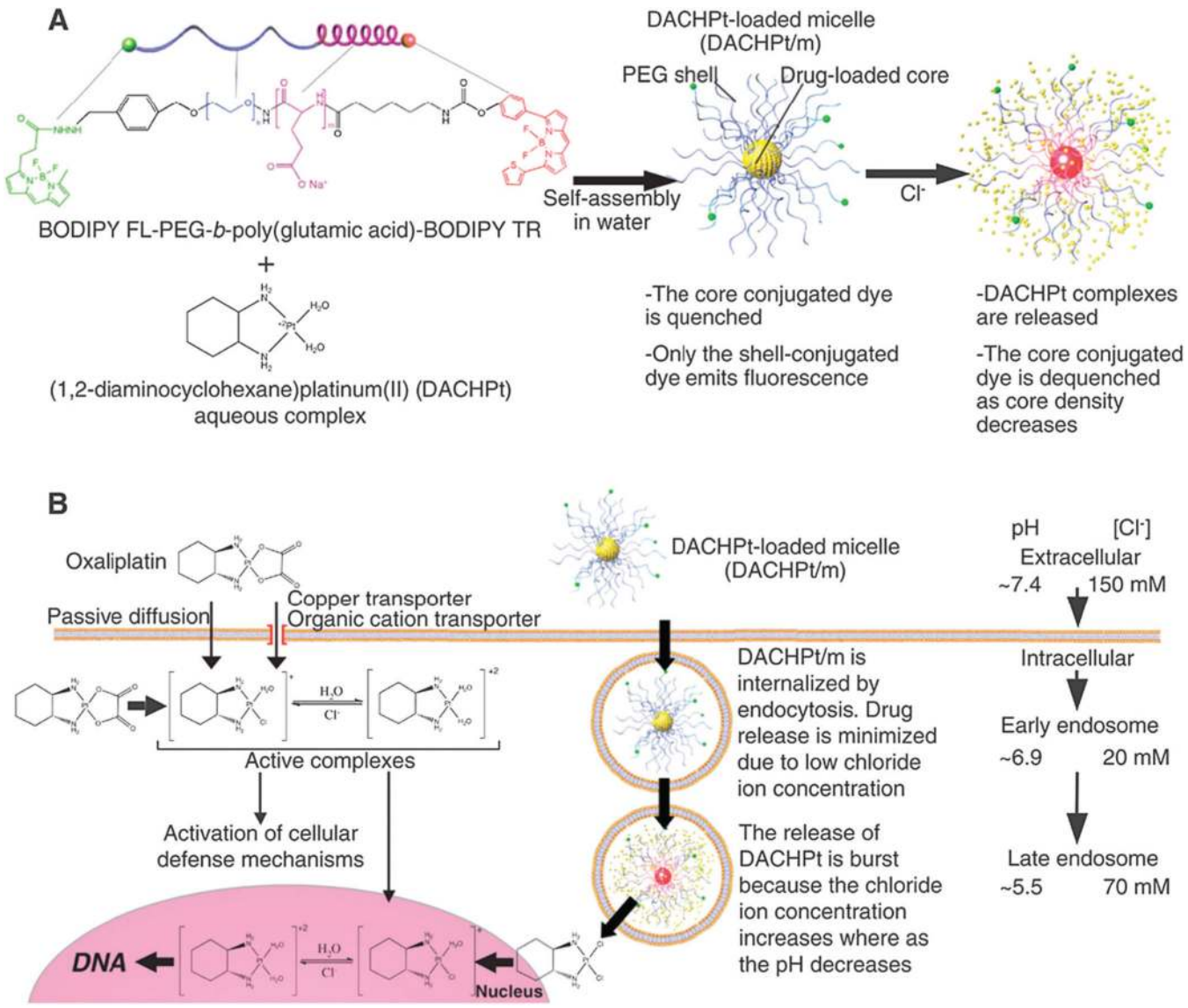

Fig. 17.

Design of fluorescent-labelled DACHPt/m (F-DACHPt/m) for visualization of localization and drug release in cancer cells: (A) F-DACHPt/m self-assembled through polymer-metal complex formation between DACHPt and boron dipyrromethene (BODIPY) FL–poly(ethylene glycol)-*b*-poly(glutamic acid)–BODIPY TR in distilled water. (B) Schematic representation of hypothetical subcellular pathways and action of DACHPt/m. Figure taken from Murakami *et al.*[423]

Table 1

Targeted NPs in clinical development

Identity	Ligand	Target	Nanoparticle	Active Pharmaceutical Ingredient (API)	Indication	Status	Reference
BIND-014	Small molecule	PSMA[a]	Polymeric	Docetaxel	Solid tumours	Phase I	19
SEL-068	Small molecule	Antigen presenting cells	Polymeric	Nicotine antigen T-helper cell peptide, TLR[b] agonist	Smoking cessation and relapse prevention vaccine	Phase I	20
CALAA-01	Transferrin	Transferrin receptor	Polymeric	siRNA	Solid tumours	Phase I	28
MBP-426	Transferrin	Transferrin receptor	Liposome	Oxaliplatin	Gastric, esophageal, gastroesophageal adenocarcinoma	Phase Ib/II	29
MCC-465	Antibody fragment	Tumour antigen	Liposome	Doxorubicin	Metastatic stomach cancer	Phase I	30
SGT53-01	Antibody fragment	Transferrin receptor	Liposome	p53 gene	Solid tumours	Phase Ib	31

[a]PSMA: prostate specific membrane antigen.

[b]TLR: Toll-Like Receptor agonist.

Table 2

Examples of preclinical targeted polymeric nanoparticles

Material	Physicochemical Characteristics (Size, ζ-potential)	Targeting Strategy	Drug/Disease or Indication	Ref.
Poly(2-methacryloyloxyethyl phosphorylcholine-*co*-butyl methacrylate) and poly(methacryloyloxyethylphos-phorylcholine-*co*-butylmeth-acrylate-*co*-methacryloyl hydrazide)	220–240 nm, 02212;2 mV	Phosphorylcholine	Doxorubicin/Cancer	153
Poly(lactic acid)-selectin conjugates	170 nm, −20 mV	Small molecule; Selectin ligand	Inflammation	154
Galactosylated-chitosan polymer	120 nm, +5 mV	Small molecule; Galactose	DNA/Various	155
Chitosan	200 nm, + 40 mV	RGD; Charge	siRNA/Cancer	156
Chitosan-PEG	150 nm, +16 mV	Antibody	Caspase inhibitor pep tide/Stroke	157
Poly(caprolactone) and poly(ethylene glycol) or poly(2-*N*,*N*-dimethylamino)ethyl methacrylate)	25–200 nm	Passive	Various	158
$(Allyloxy)_{12}$cucurbit[6]uril polymer	70–90 nm	Triggered; Reducing environment sensitive	Cancer	159
Cyclodextrin polymer	100–150 nm, +15mV	Transferrin	DNA/Cancer	160
Acetal modified dextran	250–300 nm, −5 mV to + 12 mV	Pep tide	Various	161
DNA	410 nm	Passive	Various	162
Elastin-like polypeptides	20 nm	Triggered; pH-sensitive	Doxorubicin/Cancer	163
	60 nm	RGD; T-sensitive	Cancer	164
Gelatin	250–300 nm, −20 mV	Antibody	antiCD3 mAb/Cancer	165
poly(β-amino esters)	200 nm, −5 mV	RGD	DNA/Gen therapy	166
Heparin	60 nm, −16 mV	Small molecule; Folate	Paclitaxel/Cancer	167
Hyaluronic acid	250–400 nm	Intrinsic	Cancer	168
Hyaluronic acid-ceramide/ pluronic 85	110–140 nm, −20 mV	Passive	Docetaxel/Cancer	169
Hydrophobically modified glycol chitosan	360 nm, +22mV	Charge	Cancer	170
Oligoethylene glycol pyridine disulfide nanogels	190 nm	Reducing environment sensitive	Hydrophobic drugs	171
Poly(methyldiethene-aminesebacate)-*co*-[(cholesterylox-ocarbonylamidoethyl) methylbis(ethylene) ammonium bromide]sebacate	80–180 nm, +70mV	Charge	Paclitaxel, DNA/Cancer	172
Poly(ethyleneoxide)-modified	100–150 nm, +40mV	Triggered; pH-sensitive	Paclitaxel/Cancer	173
poly(beta-amino ester)	60 nm	Triggered; pH-sensitive	Doxorubicin/Cancer	174
Modified poly(caprolactone)copolymer	120 nm, −60 mV	Small molecule; Galactose	Various	175
Poly(carboxybetaine methacrylate)	110 nm	Triggered; Reducing environment sensitive; RGD	Reducing environments	176
PEG (PRINT)	290 nm, −30 mV	Transferrin	Cancer	177
Poly(caprolactone)-	25–60 nm, −5 mV	Large peptide; EGF	Cancer	178
poly(ethyleneglycol)	70 nm, −3 mV	Pep tide	Brain	179

Material	Physicochemical Characteristics (Size, ζ-potential)	Targeting Strategy	Drug/Disease or Indication	Ref.
PEGylated Gelatin	200 nm	Passive	DNA/Various	180
Poly(methacrylic acid)	150–170 nm, −20 mV	Small molecule; Folate	Doxorubicin/Cancer	181
Poly(lactic acid)	45 nm	Peptide; RGD	Doxorubicin/Cancer	182
	70–95 nm, −30 mV to + 45 mV	Charge	Various	183
	80 nm, −25 mV	Triggered; pH-sensitive	Cisplatin/Cancer	184
Poly(D,L-lactide-*co*-glycolide)	110–190 nm	Antibody	Camptothecin/Cancer	185
	260 nm, −8 mV	Peptide	Inflammation	186
	140–180 nm, −20 mV	Peptide	Loperamide/Analgesia	187
poly(D,L-lactide-*co*-glycolide)-lipid hybrid	80–120 nm	Passive	Doxorubicin, Combretastatin-4/Cancer	188
	60 nm	Peptide	Injured vasculature	147
poly(D,L-lactide-*co*-glycolide)-poly(ethyleneglycol)	180 nm, −3 mV	Peptide; Tetanus toxin C fragment	Neurons and Neuroblastoma	189
	40–60 nm	Small molecule; Alendronate	Estrogen/Bone hydroxyapatite	190
	80–200 nm	Aptamer	Docetaxel/Cancer	191
	140 nm	Aptamer	Cisplatin prodrug/Cancer	192
	100 nm	Passive	MAPK signaling/Cancer	193
	100–120 nm, −20 mV	Peptide	Brain	194
	80 nm	Small molecule	Epigallocatechin 3-Gallate/Cancer	195
poly(D,L-lactide-*co*-glycolide)-poly(ethyleneglycol)-Aptamer	160–240 nm, −25 mV	Aptamer	Docetaxel/Cancer	196
Poly(L-lysine)	80 nm, + 1 mV	Triggered; pH-sensitive	Acidic tumours	197
Poly(lactic acid)-poly(ethyelene glycol) and poly(caprolactone)- poly(ethyeleneglycol)	20–200 nm	Ultrasound triggered	Doxorubicin/Cancer	198
Pluronic	40 nm, + 18 mV	Peptide	Cartilage	199
poly(*N*-isopropylacrylamide-*b*-methyl methacrylate)	190 nm	Triggered; T-sensitive	Prednisone/Inflammation	200
poly((1-ethoxycarbonyl)-vinyl-phosphonic diacid and poly(n-butyl acrylate)	80–120 nm	Protein; Annexin-A5	Inflammation	201
Poly(ethylene glycol)- poly(aspartate hydrazone adriamycin)	65 nm	Triggered; pH-sensitive	Doxorubicin/Cancer	89
Poly(γ-glutamic acid)-PL	115–126 nm, −20 mV	Small molecule; Galactosamine	Paclitaxel/Cancer	202
Poly(L-glutamic acid)	50 nm	Small molecule; Biotin	Doxorubicin/Cancer	203
poly(2-methyl-2-carboxy-trimethylene carbonate-*co*-D,L-lactide)	130 nm	RGD	Corneal epithelial cells	204
Poly(β-malic acid)	7–25 nm, −5 mV	Multiple; Antibody; Triggered	Antisense ON/Brain Tumour	205
	15–25 nm, −5 mV	Multiple; Antibody	Antisense oligonucleotides Hercep tin/Cancer	206
Poly(γ-benzyl-L-glutamate)-Poly(vinylybenzyllactonamide)	40–300 nm	Small molecule; Galactose	Various	207
Poly(acrylamide)	20–30 nm	Peptide	Cisplatin/Cancer	208
Poly(hydroxyalkanoates)	100–200 nm	Polypeptide	Cancer	209

Material	Physicochemical Characteristics (Size, ζ-potential)	Targeting Strategy	Drug/Disease or Indication	Ref.
Pullulan acetate/sulfadimethoxine conjugate	70 nm	Triggered; pH-sensitive	Doxorubicin/Cancer	210
Ribonucleoprotein	40–70 nm	Passive	Various	211
Styrene-maleic acid copolymers	175 nm	Zinc protoporphyrin	Cancer	212

Table 3

Novel ligands screened for targeted NP development

Category	Ligand type		Receptor	Ref.
High affinity ligands	Peptides	LHDH	αvβ3 integrin	278
		SP5-2 (TDSILRSYDWTY)	Non-small cell lung cancer	279
		NGR peptide	Angiogenic endothelial cell	280
		iRGD (CRGDK/RGPD/EC)	αvβ3 integrin and neuropilin-1, on tumour vessels	281
	Aptamers	A 10 RNA Apt	Prostate-specific membrane antigen	282
		Sgc8c DNA Apt	Protein tyrosine kinase 7 (PTK7) receptor	283
		35-mer DNA Apt	Platelet-derived growth factor	284
		88-mer DNA Apt	CCRF-CEM (Human T cell lymphoblast-like cell line)	285
	Antibodies	Monoclonal antibody A7	Colorectal carcinoma	286
		Transferrin antibody	Transferrin receptor	287
		DI17E6	αvβ3 integrin receptor	288
		2C5 antibody	nuclesome (NS)-restricted activity	289
		5D4 antibody	Prostate cancer	290
		Anti-HER2 scFv	ErbB2 receptor	291
		Anti-VCAM-1	Vascular cell adhesion molecule-1 (VCAM-1) on activated endothelial cells	292
		Anti-CD22 scFv	CD22 antigen B-cell lymphomas	293
	Other targeting molecules	Affibody *(e.g.* Anti-EGFR affibody)	EGFR	294
		Avimer	Human extracellular receptor	295
		Nanobody	Human tumour-associated carcinoembryonic antigen	273
Internalizing ligands	Peptides		PC3 cells	296
	scFv antibodies		PC3 cells	297
Transcytosis ligands	Peptides	ACTTPHAWLCG	BBB	298
		GLA and GYR		299
		TGNYKALHPHNG		194